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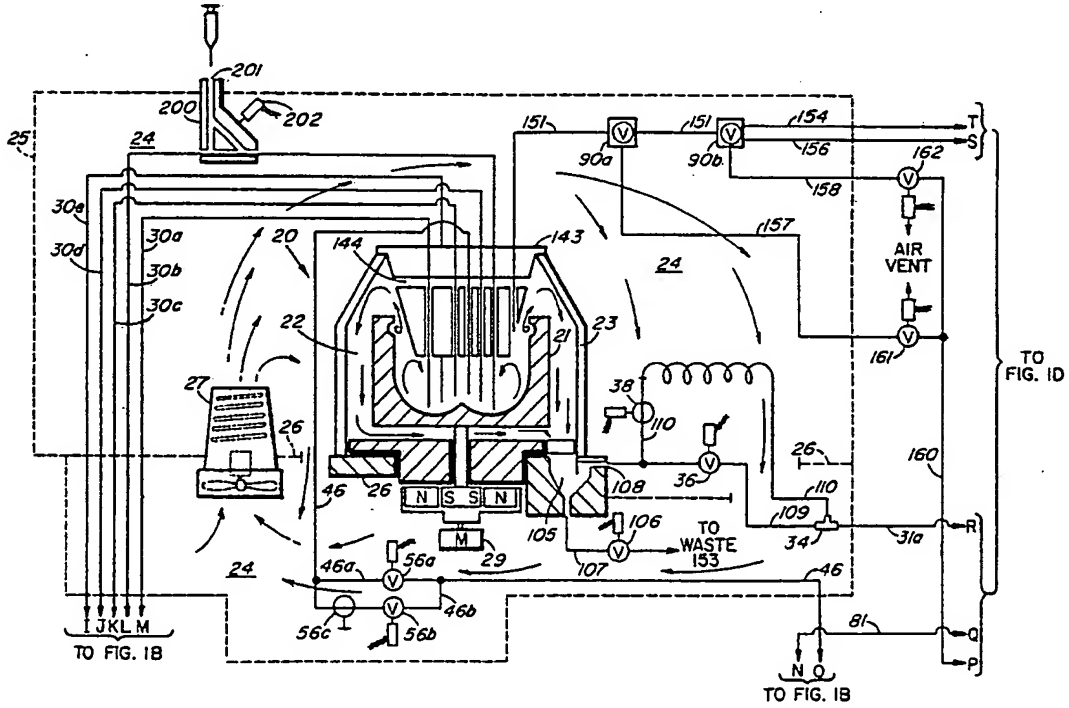

(57) Abstract

A protein/peptide sequencing system which incorporates the design changes to prevent condensation and contamination of formation in the reaction vessel and fluidic passages and controls relating thereto, improved reaction chamber cleaniness, eliminate high vacuum applications during evaporative stages of the reaction, maintain the reaction chamber enclosed to improve fresher and temperature stability and reduce contaminant formation at obtained indirectional flow of gases, vapors, liquids and wastes within the reaction chamber away from the reaction site.

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METHOD AND APPARATUS FOR SEQUENCING SMALL SAMPLES OF PEPTIDES AND PROTEINS

Field of the Invention

The present invention relates to a method and apparatus for sequencing or synthesizing an amino acid chain comprising a protein or polypeptide and in particular an improved method and apparatus for performing a sequential degradation of peptides/proteins for purposes of analyzing the constituent amino acids of the chain.

Background of the Invention

The characteristics of various proteins and peptides are determined by the particular types of amino acids making up the protein or peptide and by the sequence in which the amino acids are chemically bound in a chain-like molecular structure to form the protein or peptide. It is necessary in the analysis of a protein or peptide to accurately determine the exact order or sequence of amino acids in the chain so that a correlation between molecular structure of the protein or peptide and its function can be deduced. The most commonly used method for sequential analysis of the amino acids comprising proteins and peptides is the procedure devised by Pehr Edman between the years 1950 and 1956. A brief summary of the procedure and reference to publications describing the early developments in protein

and peptide analysis can be found in an article entitled "Sequential Analysis of Proteins and Peptides" found in the publication entitled "Fractions", No. 2, pp. 1-10, (1969). This method of analysis has become well known and commonly referred to as the Edman process. An automated method and apparatus for analysis of the amino acid sequence of proteins and peptides utilizing these teachings is described in an article authored by Edman in collaboration with Geoffrey Begg entitled "A Protein Sequenator", European Journal of Biochemistry No. 1, pp. 80-91 (1967).

In the Edman process a reagent phenylisothiocyanate (PITC) reacts with the N-terminal amino group of a peptide or protein to form a phenylthiocarbamyl (PTC) derivative. On exposure to anhydrous acid the N-terminal amino acid residue (amino group with PTC derivative) is selectively cleaved from the remainder of the peptide or protein structure in the form of a heterocyclic derivative, anilino-thiazolinone (ATZ). The cleavage reaction occurs through the attack of a sulphur atom of the PTC derivative on the carbamyl component at the first peptide bond of the amino acid chain comprising the peptide or protein, producing a cleaved ATZ amino acid derivative and releasing the residual peptide/protein. The cleaved amino acid derivative, that being the ATZ derivative is separated from the residual peptide by

extraction with an organic solvent. Since the ATZ amino acid derivative is highly unstable, it is converted through exposure to aqueous acid and heat to an isomeric phenylthiohydantoin (PTH) which is a more stable isomer.

The residual protein/peptide is then again subjected to the degradation cycle of the Edman process to sequentially and individually remove amino acid derivatives from the protein or peptide in a stable form. An analysis of each of the amino acid derivatives may be performed, such as by chromatography, to determine the kind and order of amino acids in the chain comprising the protein or polypeptide.

As shown in Fig. 3, the Edman process for sequential degradation of a peptide or protein can be described with reference to three individual steps: a coupling step, a cleavage step, and a conversion step. In the coupling step PITC (in solution with a vehicle such as heptane) is introduced. PITC reacts with the N-terminal of the amino group of the peptide or protein to form the PTC derivative. Since the PITC reacts with the amino group in its unprotonated form, the pH for the coupling reaction must be maintained in the alkaline range and preferably in the range of pH 9.0 - 9.5. Since an alkali is consumed during the coupling reaction, measures must be taken to maintain the initial pH at the

desired level throughout the reaction. This is generally accomplished by addition of a coupling buffer solution to the reactants which includes a volatile tertiary amine bearing during an allyl group, such as allyldimethylamine or Quadrol™. Furthermore, it is important to control the pH of the reactants to minimize formation of undesirable side products to the reaction which may interfere with subsequent reactions or amino acid analysis.

At the end of the coupling step the excess PITC and organic constituents of the coupling buffer are generally removed by extraction with benzene. Certain break down products of PITC such as analine and phenylthiourea are also removed in the benzene extraction. Diphenylthiourea which is another side product is poorly soluble in benzene and must be removed by a second extraction with a polar organic solvent such as ethyl or butyl acetate. Water introduced by the coupling buffer must also be removed, for example by lyophilization. The vessel in which the reaction is conducted is then purged, such as with an inert gas or by evacuation, to remove remaining undesired vapors. The PTC derivative of the amino acid is dried and prepared for cleavage.

In the cleavage step anhydrous acid, such as trichloroacetic acid or N-heptafluorobutyric acid, is

used to dissolve the first peptide bond attaching the first amino group with the PTC derivative to the protein or peptide, resulting in the ATZ derivative of the amino acid being formed and selectively cleaved. The cleavage reaction occurs very rapidly in the presence of a strong shift in pH of the reactive environment caused by introduction of the anhydrous acid. After completion of the cleavage reaction, remaining acid is removed. The cleaved ATZ derivative is separated from the residual protein or peptide by extraction with butyl chloride or ethylene dichloride. The residual solvents remaining in the reactive solution are also removed, leaving the peptide or protein in prepared form for another coupling and cleavage cycle.

The coupling and cleavage steps must be performed in an oxygen free environment. Presence of water (oxygen) can cause aqueous acid formation which may result in unspecific hydrolytic peptide bond cleavage along the amino acid chain forming the peptide or protein. Separation of the cleavage step from the conversion step of the Edman process is thus critical to permit the cleavage reaction to be carried out under anhydrous conditions in order to avoid nonspecific bond cleavages caused by aqueous acid. Furthermore, oxygen in the reaction environment must be avoided to prevent desulfuration of the phenylthiocarbamol (PTC) derivatives

which would convert them to a phenylcarbamyl form. The phenylcarbamyl form cyclyzes only under severe conditions so for practical purposes the peptide or protein would be blocked from further participation in sequential degradation.

The extraction solution containing ATZ derivative is placed in a second reaction vessel and the residual acid and solvents evaporated to substantially dry the compound. The ATZ derivative is thus prepared for the conversion step.

In the conversion reaction aqueous acid is used to convert the ATZ derivative to an isomeric PTH derivative which is highly stable and lends itself to analysis of the base amino acid by an appropriate method such as gas or high pressure liquid chromatography. Partial conversion of the ATZ derivative to a thiohydantoin may have already occurred during the cleavage extraction and evaporation procedures of the preceding steps. Hydrochloric acid or tetrafluoroacetic acid (TFA) is a suitable aqueous acid for obtaining conversion. The PTH derivative is then extracted for analysis. Most PTH derivatives of an amino acid may be extracted from the aqueous solution with ethyl acetate, or dried and re-dissolved in a suitable organic solvent such as acetonitrile.

A typical apparatus for performing the described sequential degradation steps of coupling cleavage and conversion is described in numerous publications, the most relevant of which are Edman and Begg,"A Protein Sequenator", European Journal of Biochemistry, No. 1, pp. 80-91 (1967); Niall, "Automated Edman Degradation: The Protein Sequenator", Methods and Enzymology Vol. ___ , pp. 942-1011 (1972) and many patents, the most relevant of which are as follows:

"Apparatus for Sequencing Peptides and Proteins", Gilbert, U. S. Patent No. 3,892,531;

"Method and Apparatus for Analyzing Proteins", Sjoquist, U. S. Patent No. 3,645,689;

"Peptide or Protein Sequencing Method and Apparatus", Dreyer, U. S. Patent 4,065,412;

"Process and Apparatus for Conversion of ATZ to PDH Amino Acid Derivatives of Proteins", Bonner et al., U. S. Patent No. 4,155,714;

"Apparatus for Performance of Chemical Processes" Good et al., U. S. Patent No. 4,252,769.

"The procedure for sequencing residues from proteins follows the now familiar pattern, (1) sample is dissolved in a nonvolatile buffer, the alkaline component of which is Quadrol^{TM}, (2) PITC couples with the N-terminal alpha amino group, (3) benzene precipitates the protein and carries off the excess PITC, (4) ethyl acetate washes out the Quadrol, (5) anhydrous heptafluorobutryic acid cleaves off the N-terminal amino acid as the diazolinone amino acid (ATZ) and (6) butyl chloride extracts the cleaved product and takes it to the fraction collector. With smaller peptides, the various washing cycles become more critical, and some differences in procedure are indicated. For one, the sample is dissolved in a volatile buffer, dimethylallylamine, which is subsequently removed by a vacuum, instead of by use of ethyl acetate in step 4, or both."

A system representative of current apparatus for peptide and protein sequencing is the Beckman Model 890^{TM} Protein and Peptide Sequencer available from Beckman Instruments, Inc., Spinco Division, Palo Alto, California. Features the novel aspects of this instrument are described in the following patents and patent applications:

"Apparatus for Automatically Performing Chemical Processes", Penhasi, Patent No. 3,725,010;

"Process for the Sequential Degradation of Peptide Chains", Penhasi et al., Patent No. 3,717,436;

"An Automated Chemical Conversion Unit in a Peptide Protein Sequenator", Ohms et al., Patent Application Serial No. 500,670.

In an Edman or Beckman type system, as is described in the above referenced patents, all of the chemical reactions and functions are performed in a continuously spinning cylindrical cup which comprises the reaction vessel. [In fluid communication with the reaction cup are means for introducing and removing gases (e.g., nitrogen) and liquids (i.e., reagents, solvents). The chamber within which the reaction cup is positioned is atmospherically sealed to permit evacuation and insulated to provide a constant and uniform temperature and pressure critical for the reaction. It is necessary to provide a properly sealed drive mechanism for the spinning reaction cup within the chamber to adequately obtain a vacuum environment, such as through use of a magnetically coupled drive unit which is completely sealed.]

Some described systems require the conversion step to be done manually after the ATZ reaction product has been

suitably prepared and dried while others provide intermediate reaction vessels for automatically performing the conversion of the ATZ derivative to a PTH derivative form.

As the sample is introduced the protein spreads as a thin film over the surface of the lower half of the cup. Reagents are introduced into the cup so as to flow over the film of protein. The movement of one thin film over another is ideal for rapid solution, reaction and extraction processes. Solvent extractions are carried out by allowing the liquid to flow up over the surface of the dry protein to the top of the cup where it reaches a groove formed in the interior surface of the cup adjacent to the top edge where the extractant can be scooped up and removed. Spinning of the reaction cup also helps reduce the tendency of contained materials to boil during evaporation of the liquids under application of vacuum.

Current peptide and protein sequencers continue to exhibit less than desirable performance, reliability and convenience for the user. Frequent and extensive cleaning is generally required of the reaction chamber reaction cup and associated fluid transfer systems even during the progress of a long chain peptide/protein degradation. These cleaning requirements often result in contaminants being permitted to remain which affect the

performance of the sequencer. Furthermore, mistakes are often made in reassembly of portions of the system which must be disassembled for cleaning. Opening of the reaction chamber permits the sequencer system to cool, requiring lengthy periods of time to regain temperature equilibrium which may not be able to be accurately duplicated. These problems have been further exacerbated by a substantial reduction in the volume of sample utilized to perform a sequential degradation methodology. Original instruments were designed to operate in the 100-300 nanomole sample level while current demands have reduced sample volume to a 20-200 picomole volume, a reduction of approximately 1,000% in sample volume. Such sample size reductions have substantially increased contaminant and reaction interference in the formation of the derivative product, making accurate determination of a peptide or protein amino acid sequence more difficult and less reliable.

Furthermore, interferences which have been known and studied, such as the presence of particular contaminant materials within the reaction chamber, have been misidentified as to source resulting in incorrect and inappropriate corrective measures in instrument design and operation. For example, it has been recommended by numerous sources to provide high vacuum evacuation systems for purge of the reaction chamber,

including contaminant condensation devices (e. g. cold traps) within the fluidic communication lines between the chamber and vacuum source. Present discoveries and research have found these devices unnecessary and in fact, their inclusion to be detrimental to system performance, efficiencyand reliability while increasing system expense.

Additionally, inappropriate positioning of fluid communication lines and control valving and/or failure to provide adequate temperature stability for them, result in continual distillation/condensation occurrences which lead to formation of contaminants which interfere with the reaction or which can cause flow restrictions. Due to the extensive usage of volatile reagents and the back to back base-acid environments found in the coupling and cleavage steps, nonvolatile and insoluble salts and other residues are often formed in places which are impossible to clean by automated methods and even difficult to clean after disassembly of the related components. Accumulation of these contaminants over a period of time can lead to substantial interference by them with the conducted reactions and thus analysis of the peptide or protein amino acid sequence.

Summary of the Invention

The present invention provides an automated protein

and/or peptide sequencer which avoids or minimizes the described limitations and difficulties of known instruments. The disclosed sequencer is capable of sequentially degrading proteins and/or peptides containing large numbers of amino acid units with improved accuracy, reliability and convenience.

A protein/peptide sequencing system is provided which in addition to the present invention, comprises many of the features taught in the above referenced patent, 3,275,010 and patent application serial No. 500,670. Invention is disclosed by incorporating design changes to attain the following objectives:

(a) maintain a constant temperature and pressure environment for the degradation reaction and sensitive fluidic portions of the sequencer system to assure repeatability in protein and/or peptide sequence degradation;

(b) eliminate a need to open or enter the system reaction chamber, e.g., for cleaning, thereby improving pressure and temperature stability by eliminating change due to entry into the chamber and eliminating the possibility of contamination from the outside environment;

(c) maintain the reaction cup in which the degradation reaction is performed at a temperature which is cooler than any other portion of the reaction chamber to prevent condensation of reagent or solvent vapor on other portions of the chamber, thus reducing the possibility of contaminant formation within the reaction chamber;

(d) assure surfaces of the reaction chamber maintained in a state of cleanliness to retard formation of condensate and contaminant; and eliminate or minimize areas of the reaction chamber in which contaminants and residual materials can form or lodge to improve chamber cleanliness;

(e) obtain a uniform and unidirectional flow of gases, vapors and liquids within the reaction chamber and away from the reaction site within the reaction cup to improve environmental conditions for the reaction, and to assure contaminants and residual substances are carried away from the reaction for removal from the chamber, to assure reversal of flow does not carry back substances which may result in increased contaminant formation and to prevent reaction by-products from reentering the reaction cup;

(f) provide inert gas flow to assist evacuation and purge of reaction chamber;

(g) provide a reaction chamber drain for removal of waste gases, vapors and liquids;

(h) provide an automated method of cleaning the entire reaction chamber without requiring entry or disassembly of the sequencer system components in the reaction chamber, in which waste material is flowed away from the reaction vessel to prevent contamination when the degradation reaction takes place;

(i) automate sample introduction to the reaction cup and embody all necessary system apparatus and/or components used by the system for sample introduction within a temperature controlled environment to prevent formation of condensate (such that cross contamination of sample is prevented);

(j) position all reagent/solvent/effluent valves within a temperature controlled environment to eliminate formation of condensate and thus contaminants which may effect the reaction or fluid flow;

(k) arrange reagent and solvent reservoir venting in a manner to prevent interaction of vented vapors and

the formation of contaminants;

(l) eliminate all unnecessary contaminant collecting components from fluidic lines communicating with the reaction chamber;

(m) add of particulate straining/filtration for all reagent and solvent reservoirs;

(n) reduce the number of reagents required for degradation reaction;

(o) eliminate all high vacuum evacuations of the reaction environment, i.e., the reaction, etc.

Description of the Drawings

Figs. 1A, 1B, 1C and 1D are schematic representations of the automated protein/peptide sequencer presented herein;

Fig. 2 is a diagrammatic illustration of the constant pressure delivery system for reagent and solvent used in connection with the respective reservoirs;

Fig. 3 is a schematic representation of the three steps of the degradation reaction; the coupling step, the cleaving steps and the conversion step;

Best Mode

An automated protein/peptide sequencer embodying the present invention is described with reference to Figs. 1A, 1B, 1C and 1D. A reaction cell and chamber assembly generally indicated by reference numeral 20 includes a reaction cell or cup 21 disposed within a reaction chamber 22. The reaction chamber 22 is formed by a generally cylindrical enclosure 23. Reaction cell assembly 20 is in turn positioned within the heated chamber 24 defined by an enclosure, schematically indicated by broken line 25, which is fabricated from suitable insulating materials, such as plexiglas or phenolic to maintain thermal control and efficiency. Heated chamber 24 is preferably divided into upper and lower portions by a base plate 26 which serves as a baffle. Base plate 26 extends horizontally across the heated chamber as indicated. Thermal control means 27 is located within the chamber 24 to maintain the reaction cell assembly 20, the drive assembly, and all contained system elements contained within the heated chamber at a predetermined and constant temperature.

The reaction cup 21 is secured to the top of a rotatable shaft 28 which is driven by a multiple or variable speed motor 29. The speed of the motor 29 is preferably adjusted between two predetermined speeds

referred to hereinafter as high or low speed for purposes which will be discussed later.

By spinning the reaction cup 21 the various solvents, reagents and solutions, form a thin film over the interior surfaces of the glass cup due to the associated centrifugal force. Spreading the various sample reagents and solvents as thin films on the inside wall of the cup one over another increases their interaction and speeds and enhances the degradation reactions and extractions performed therein. It additionally presents a larger stabilized surface area which may be more rapidly dried under reduced pressure conditions.

The reagents and solvents used throughout the degradation reactions are introduced into the reaction cup 21 by way of four separate feed lines shown schematically by lines 30a, 30b, 30c and 30d, each of which extends through the top of the enclosure 23 and extends downwardly toward the bottom of the reaction cup 21. The feed lines 30a-30c terminate slightly above the bottom of the cup 21 with sufficient clearance above the bottom of the cup that the liquid flow is not unduly restricted. The feed lines 30a-30d are arranged in a circle around the reaction cup's longitudinal axis to facilitate the smooth and uniform introduction of the

reagents and solvents into the spinning cup 21. Each of the feed lines 30a-30c is approximately 75% contained (over its total length) within the heated chamber 24 so that fluids passing through these feed lines are heated to and maintained at the predetermined and constant temperature of the chamber 24. Additionally, flow control valves directing flow of solvent and reagent through the feed lines 30a-30c are preferably contained within heated chamber 24 or another temperature controlled region of the sequencer to similarly assure fluids which they direct are heated to and maintained at the desired temperature. All feed, effluent and drain lines are fabricated from a suitable noncorrosive material, such as Teflon.

A sample injection valve 200 is provided in feed line 30b, which is positioned within the heat chamber 24 exposing only a sample receiving port 201 outside of the chamber. The sample receiving port 201 is adapted to receive a syrine or pipettor, etc., for introduction of sample solution. The sample receiving port 201 is opened to the feed line 30b through control of vale 200 by a suitable solenoid actuator, however, the valve may be controlled manually. It is particularly beneficial however, to provide automated means for closing the sample valve receiving port 201 to the feed line with which it communicates after sample has been introduced.

Temperature control of the sample valve 200 to the temperature at which the chamber 22 is maintained assures condensate and/or residue will not form within the valve, thus blocking flow or introducing contaminants into the reactants. The sample valve 200 (as are the later described effluent and selector valves 90a and 90b, respectively) is obtainable from the Rheodyne Company, as a Model 5302 Valve having a Model 5300 Actuator.

The reaction cup 21 is preferably a cylindrical cup fabricated from a suitable noncorrosive material such as borosilicate glass. An annular groove 101 is formed around the inner surface near the top edge of the cup. The cup 21 preferably has an inside diameter of approximately 25 millimeters. The height of the inside of the cup measured from the bottom to the annular groove 101 is approximately 31 millimeters. The annular groove 101 is approximately 2.5 millimeters deep as measured from the inner surface of the cup and approximately 6 millimeters wide. Reaction cup dimensions can be proportionally reduced if system size reduction is desired. It should be noted that the deeper the annular groove, the better scooping action of an effluent line having a pick up positioned within the group will be performed. The interior wall surface around the bottom of the cup 21 is rounded to approximately a 6 millimeter radius to minimize the sample thickness at the bottom of

the cup, thus enhancing distribution of the sample for the chemical reactions, improving the washing action of the solvent and wash solutions and preventing the sample from becoming lodged in the corner of the cup. This cup configuration also prevents boiling of solvents, reagents or solution during vacuum application periods in the drying cycles which could be initiated at sharp corners. The bottom of the cup interior is constructed flat and tangent to the corner radii, with a centrally located conical protrusion approximately 3 millimeters high and 5 millimeters in diameter at its base. This protrusion prevents the accumulation of various residues at the center of the cup which lies at a point of zero centrifugal force.

The reaction chamber 22 is defined by enclosure 23 having its lower open end mounted to base plate 26 in a manner that discourages disassembly, such as by a plurality of screw attachments (not shown). The enclosure 23 is sealed to the base plate 26 to permit formation of a vacuum within or pressurization of the chamber 22 defined therein. The enclosure 23 also includes a centrally located top opening 142 for receiving a support plug 103.

The support plug 143 is generally conical in shape and preferably fabricated of an inert fluorocarbonpolymer

such as Teflon™ (from E. I. DuPont de Nemours and Company). Other suitable shapes may however, be utilized for the construction of the support plug 143. An O-ring seal contained within a groove formed about the top opening 142 of the enclosure 23 serves as a fluid seal between the plug 143 and the enclosure 23. To enable the sequencer to operate with highly volatile reagents it is essential that the total unoccupied volume of the reaction chamber 22 be kept to an absolute minimum and preferably below 250 cc. This permits rapid equilibration of temperature and vapor pressure, permits rapid chamber evacuation or pressurization and minimizes evaporative losses, thereby permitting the sequencer to easily handle highly volatile reagents as well as nonvolatile reagents. Furthermore, minimized chamber volume facilitates control of gas and vapor flow patterns with respect to the reaction cup 21. This is accomplished by the body of the support plug 103 which extends into the interior of the enlosure 23, i.e., chamber 22, and into the interior of the reaction cup 21.

The support plug 143 is inserted downwardly into the reaction chamber 22 and partially into the reaction cup 21 through opening 142 and sealed to the enclosure 23. Insertion of the plug 143 into the reaction cup 21 substantially reduces the volume within the reaction cup 21 and controls the flow of contents held within the

reaction cup 21 into the reaction chamber 22. Such an arrangement minimizes turbulence between the reaction chamber 22 and reaction cup 21 and for practical purposes eliminates any pumping action of vapors between the cup interior and the reaction chamber by the spinning reaction cup. For practical purposes, the vapor circulation is contained within the reaction cup 21 minimizing vapor loss through evaporation. Additionally, the support plug 143 substantially reduces the volume within the reaction chamber 22 and reaction cup 21 to improve thermal response and fluid flow characteristics therewithin. Positioning of the support plug 143 in such a manner also minimizes contact of any undesirable vapors with various surfaces within the reaction chamber 22 to reduce the possibility of condensation and residue formation.

The nitrogen feed line 46 is located in the center of the support plug 143 along its longitudinal axis and that of the reaction cup 21 while the feed lines 30a-30c are arranged in a circular pattern about the nitrogen feed line 46. Positioning of the nitrogen feed line in the support plug 103 which extends into the interior of the reaction cup 21 permits introduction of the pressurized nitrogen into the cup interior to flow nitrogen (or other inert gas) over the contents of the

cup during application of vacuum to greatly improve the vacuum drying efficiency within the cup 21.

Effluent line 151 also enters the reaction cup 21 through support plug 143 and extends towards the inner wall of the reaction cup 21 with its tip disposed in the groove 101. The effluent line 151 has a pick up portion facing in opposition to the direction of rotation of the reaction cup 21. A very narrow clearance between the groove 101 and the pick up of effluent line 151 is provided for efficient fluid removal from the cup 21. Scooping of the reagent products and byproducts and extracting solvents is by the effluent line 151 assisted by the momentum of the impinging fluids on the pick-up effluent line 151, as well as by nitrogen pressure introduced within the reaction cup when nitrogen is fed through line 46. Effluent line 151 may be externally adjusted, along its longitudinal axis and angularly, to orient its pick up in an optimum position within the groove 101 for most efficient withdrawal of fluids.

The support plug 143 comprises a plurality of longitudinal channels to receive and support solvent reagent feed lines 30a-30c, nitrogen feed line 46, and effluent line 151. A wash channel 144 is formed laterally through the support plug 143 and communicates fluidically with each of the longitudinal channels also

formed in the plug 143. Feed and effluent lines are sealed in their respective longitudinal channels above wash channel 144 to provide support and prevent vapors, chemicals or nitrogen from entering in or escaping through the space between the channel wall and line. This assures that contaminant or condensate does not form and accumulate in these spaces. The longitudinal channels are sized larger than the encased feed and effluent lines below wash channel 144 to permit nitrogen entering through nitrogen feed line 46 and wash solution to enter between the channel wall and its respective line to wash out any residue or contaminant formed therebetween. The wash channel 144 thus provides means for inert gas and wash fluid to enter and flow down through the cavity between the solvent/reagent feed lines, etc., and the channels through which they extend to assure that all contaminant material is removed therefrom during a nitrogen flow or wash cycle, thus preventing any contaminants from remaining and entering the interior of the reaction cup 21 during the degradation reaction.

The support plug 143 fits somewhat loosely within the opening of reaction cup 21 leaving an annular clearance between the plug 143 and the upper edge of the reaction cup 21 so while fluids in the spinning cup 21 are substantially contained, pressurized nitrogen and

wash solution fed into the cup 21 may escape and flow into the reaction chamber 22 for purge/wash of the chamber nitrogen and wash solution are removed through drain 145 and waste line 147. This also enables vacuum applied to the reaction chamber 22 to evacuate the interior of the reaction cup 21 and cup/chamber evacuation to be assisted by a flow of nitrogen to the cup 21 and through the chamber 22 as supplied by nitrogen feed line 46 opening to the bottom of reaction cup 21.

Support plug 143 may be provided with a temperature probe such as a thermistor encapsulated in a glass rod, for continuously monitoring the temperature within the interior of the reaction cup 21.

As previously described the determination of the amino acid sequence in proteins and/or peptides is based on the three step degradation (phenylisothiocyanate) reaction. It has been found that the phenylthiocarbamyl (PTC) group formed during this process is easily desulfurized by oxidation in the presence of oxygen converting it to a phenylcarbamyl form and terminating the degradation process. This problem was referred to above with regard to application of reduced levels of vacuum throughout the conversion and cleavage steps. For practical purposes desulfuration blocks the coupling site of the peptide/protein from further participation in the

degradation reaction since the thiazolinone derivative whose formation is essential to a successful conclusion of the sequence can no longer be formed. As a consequence, the degradation process must be carried out in an oxygen free atmosphere. This is accomplished by evacuation of the reaction chamber 22 and/or by filling the reaction chamber 22 with a suitable inert gas, such as nitrogen, helium, neon, argon, etc.

Evacuation of the reaction chamber 22 and reaction cup 21 is provided by means of a vacuum pump 31. Vacuum pump 31 is connected to the reaction chamber 22 through a vacuum line 31a which communicates through vacuum port 108 at a lowermost portion of the chamber 22 adjacent to the drain port 145. Vacuum pump 31 is connected to the reaction chamber 22 through a main vacuum line 109 and restricted vacuum line 110.

In practice the vacuum pump 31 is continually operated. Consequently, vacuum lines 109, and 110, each have a solenoid actuated flow control valve 36, and 38, respectively, which control application of a select vacuum to the reaction chamber 22. When applying a vacuum to the reaction chamber 22 solenoid valve 38 is opened first to communicate vacuum to the reaction chamber 22 through the restricted vacuum line 110 This serves to gradually apply vacuum and prevent the liquid

contained within the reaction cup 21 from boiling due to sudden application of vacuum. As the reaction chamber 22 and reaction cup 21 are partially evacuated, solenoid actuated valve 36 is opened to connect the vacuum pump 31 directly to the reaction chamber 22 through vacuum line 109 which serves to apply unrestricted vacuum during the remaining time period of each evacuated drying step. When vacuum is applied to the reaction chamber 22 and reaction cup 21 to dry reaction product and remove residual vapor, purge flow is assisted by introduction of nitrogen through the cup and the reaction chamber, from which residuals are removed.

It should be noted here that the level of vacuum applied to either the reaction chamber 22 or to the reaction cup 21 has been substantially reduced from prior art teachings, and is preferably limited to 200-500 mm of Hg as a maximum vacuum application. Such has been found to be adequate for obtaining rapid drying of sample or reaction product, etc., when coupled with flow of nitrogen (or other inert gas) through the reaction cup 21. Reduced vacuum application assures the bound protein sample will not be over dried reducing post-coupling wash and post-cleavage fraction extraction efficiency and reduces the risk of oxidative desulfuration of the derivative of the peptide protein, by oxygen or oxidants contained in the reagent, .

Vaccuum pump 31 may also be connected to the fraction collector 155 through vacuum line 164 which includes solenoid operated flow control valve 39 to provide for evacuation of the fraction collector when reaction product is contained therein. However, vacuum application to the fraction collector 155 may also be provided by second vacuum pump 32 through vacuum line 164' including a flow control valve 39'. The primary function of second vacuum pump 32 is to provide vacuum for the operation of flow control valves for solvent/reagent delivery and or for effluent/waste removal.

Nitrogen is provided by two commercially available cylinders of compressed nitrogen 44 and 45. Nitrogen is fed from both of these cylinders to the reaction chamber 22 by way of nitrogen feed line 46, through spur lines 46a, 46b, which communicates with the interior of the reaction cup 21. Nitrogen feed line 46 is in turn connected to nitrogen cylinders 44 and 45 by a distribution manifold 47, branch lines 49 and 50 and pressure regulators 48 and 48A, respectively. A pressure switch 51 is located at the junction of branch lines 49 and 50 to monitor the pressure of the nitrogen being supplied by each of the nitrogen cylinders and switches from one nitrogen cylinder to the other when the nitrogen

supply being provided falls below a predetermined pressure. For instance, assuming that nitrogen is initially being supplied by cylinder 44 and is switched from this supply to nitrogen cylinder 45, pressure switch 51 upon sensing a predetermined low pressure provides a signal which simultaneously closes the solenoid actuated valve 52 shutting off the nitrogen supply from cylinder 44 and opens the solenoid actuated valve 53 connecting manifold 47 to nitrogen cylinder 45. When both nitrogen cylinders are exhausted the control system shuts the sequencer system down. Each branch line 49 and 50 is connected to a separate manually actuated vent valve 54 which is open following the installation of each nitrogen cylinder to vent any air and other contaminants which may be inadvertently introduced into these lines during the installation of the nitrogen cylinders.

Nitrogen feed line 46 is branched into two individual spur lines 46a and 46b which are later recombined into a common feed line 46. Each of the spur lines 46a and 46b contain solenoid actuated valves 56a and 56b respectively, which are positioned inside heated chamber 24 of the reaction cell and chamber assembly 20 for controlling the flow of nitrogen into the reaction cup 21 and reaction chamber 22. Spur line 46b also contains a manually adjustable needle valve 56c which may be used to adjust the flow rate of nitrogen gas. Thus

when solenoid valve 56b is actuated to provide nitrogen flow only through line 46b, the flow rate may be adjusted to a level less than that occurring through line 46a when valve 56a is actuated. This provides for two selectable nitrogen flow rates for introduction of nitrogen into the reaction cup 21 and reaction chamber 22, as desired. A greater number of branches, valves, etc., may be provided if greater flow rate selection is desired. Finally, a hand operated shut-off valve 57 is located in nitrogen feed line 46 between pressure regulator 55 and solenoid actuated valves 56a, 56b, as a safety measure to isolate the nitrogen supply cylinders 44 and 45 from the reaction chamber 22 when the protein/peptide sequencer is not in operation. Of course, when the sequencer is being operated shut off valve 57 remains open.

In addition to purging the interior of reaction chamber 22 of oxygen and water vapor, the nitrogen supply may be used to maintain the reaction chamber 22 and the reservoirs containing various reagents and solvents at predetermined pressures. To this end the hand operated pressure regulation valves 48 and 48A are inserted between the junction of branch lines 49 and 50 and nitrogen cylinders 44 and 45. For example, the pressure regulation valves 48 and 48A preferably reduce the pressure of the nitrogen to around 60-90 psi and enters the distribution manifold 47. The pressure of the

nitrogen may be further reduced before it enters the reaction chamber 22 by means of hand operated pressure regulator 55 positioned in the nitrogen feed line 46 at the outlet side of the distribution manifold 47. For example, nitrogen pressure in the reaction chamber 22 is preferably controlled to approximately 60 millimeters of Hg (1.16 psig). Of course, other pressures may be used depending upon reaction parameters and design of the system flow components.

The reagents and solvents used throughout the degradation reactions are stored in a plurality of reservoirs 58, each of which may typically take the form of a bottle fabricated from a suitable noncorrosive material such as borosilicate glass or plastic. For purposes of description the reservoirs are designated R1, R2, R3, R4, R5, and S1, S2, S3 and S4 for the reagent (R) and the solvent (S) each contains. Each of the reservoirs 58 is connected to nitrogen supply through connection with manifold 47 both to remove dissolved oxygen from the reagents and solvents and to maintain the reservoirs 58 in an oxygen and water free environment at a constant predetermined pressure to provide motive force to drive the reagents or solvents through their respective feed lines 30 into the reaction cup 21. Nitrogen is fed to the solvent reservoirs S1-S4 by way of distribution manifold 47, a nitrogen feed line 59, and

common distribution manifold 60. Manifold 60 supplies four pressure lines 61, 62, 63 and 64, each of which is connected to one of the solvent reservoirs S1-S4, respectively. Cross contamination of solvents is not a problem with the sequencer system operation so a common solvent distribution manifold is utilized to reduce system complication and expense. A pressure regulator 65 is placed in nitrogen feed line 59 to control the pressure of nitrogen in manifold 60 and delivered to the solvent reservoirs. Preferably, nitrogen pressure is reduced to around 140 millimeters of Hg (2.7 psi).

Each of the pressure lines 61, 62, 63 and 64 from the distribution manifold 60 includes a solenoid actuated valve 66a, 66b, 66c and 66d, respectively, for selectively controlling the introduction of nitrogen into solvent reservoirs S1-S4 and to otherwise isolate the respective reservoirs when awaiting use.

In a manner similar to that applied to the solvent reservoirs, the reagent reservoirs R1-R5 are also connected to the nitrogen supply through connection with distribution manifold 47. However, cross contamination between reagents can critically affect performance of the degradation reactions and to prevent cross contamination of the various reagents, nitrogen is fed to each of the reservoirs R1-R5 by way of a separate nitrogen pressure

line 67a, 67b, 67c, 67d and 67e, respectively, each of which is connected to an individual outlet of distribution manifold 47 as indicated by the designations R1, R2, R3 R4 and R5. The introduction of nitrogen into the reagent reservoirs R1-R5 is controlled by means of solenoid actuated valves 66a, 66b, 66c 66d and 66e, positioned in each nitrogen pressure line 67a-67e, respectively. A pressure regulator 70, the same as regulator 65, is provided in each nitrogen feed line 67 to control the pressure of nitrogen being fed to the reagent reservoir. Preferably, the pressure is reduced to around 100 to 200 mm of Hg (1.9 to 3.87 psig). Regulators 70 also serve as a means for independently controlling the individual flow rates of each reagent by varying the pressure applied to each reagent reservoir independently of one to another. Each of the reservoir nitrogen pressure lines 67a, 67b, 67c, 67d and 67e, and the nitrogen feed line 59 include a hand operated shut-off valve 57 which is closed when the sequencer is not operating and remains open during operation of the sequencer. Finally, a suitable pressure gauge 71 may be associated with each nitrogen feed or pressure line to monitor the pressure of the nitrogen flowed therethrough.

Both the reagent and solvent reservoirs 58 are selectively vented to the atmosphere via vent lines 95 connected to vent manifolds 72 and 73. A plurality of

solenoid actuated valves 74a, 74b, 74c 74d, 74e, 74f, 74g, 74h, and 74i are provided, with one valve located in each of the reservoir vent lines 95 to selectively connect each reagent and solvent reservoir to the atmosphere, or otherwise isolate it. In addition, a hand operated metering valve 75 is provided in each of the vent lines 95 which is manually adjusted to provide a continuous nitrogen flow through each vent line when its associated solenoid valve 74 is open. The flow may be in the range of 30-50 cc. per minute.

The arrangement or order of the connections of vent lines 95 to the vent manifolds 72, 73 is a significant feature of the present invention. Vent manifolds 72, 73 are continually purged by a controlled flow of nitrogen entering through nitrogen feed line 113 and exiting with extracted vapors through line 114 to an exhaust fan 41 (e.g., upwardly through the vent manifold shown in Fig. 1B). Thus, vent lines 95 connected to the manifolds 72, 73 downstream of the entering nitrogen are exposed to extracted vapors in the gas flow from the vent lines 95 connected to the manifolds upstream. The vent lines 95 are thus arranged in order of connection with vent manifolds 72 and 73 such that vent lines from the reagent reservoirs containing an acid type reagent are positioned upstream of all other vent lines 95, or in other words, at the end of the vent manifold 72 at which nitrogen

enters from feed line 95. The openings of the vent lines 95 for the acid based reagent tend to clog with residue formed from vapors exiting other reagent/solvent containers, especially those from base type reagents, when positioned downstream. This causes unreliable flow control of reagents from those acid based reagent reservoirs, due to inaccurate pressure control. With the acid reagent lines positioned upstream of the other vent lines this problem is eliminated since the vent line openings are never exposed to residue forming vapors from other reagents or solvents. Furthermore, the vent line for the reagent container 58 containing base solution is preferably connected to vent manifold 72, 73 as far downstream as possible, i.e., the last position, so that vapors from the basic solution will be immediately directed through the vent line 114 leaving the manifold 73 to the exhaust fan 41. The preferred order of vent line connections to the vent manifolds 72, 73 from entry of purging nitrogen gas to its exit, is, using reagent nomenclature which will later be identified as to specific chemicals, R3, R5, R1, R4, S1, S2, S3, S4, R2, as shown in Fig. 1B.

Each of the reagent reservoirs R1-R5 and the solvent reservoirs S1-S4 are connected within an outlet line 76a, 76b, 76c, 76d 76e and 96a, 96b, 97c and 96d, respectively for reagent reservoirs and solvent

reservoirs. These lead through flow control valves 77-80 to reagent and solvent feed lines 30a, 30b, 30c, 30d and 30e. Flow control valves 77, 77', 78, 79, 80 are vacuum actuated valve assemblies with each valve assembly including in general two valve units identified as 103 and 105 mounted on a common manifold. These valves assemblies are described in detail in referenced U.S. Patent No. 3,725,010. Reagent is fed to valve unit 105 of each valve assembly by way of the associated connecting line 76a-76e, while solvent is fed to the valve unit 103 of each valve assembly via the associated connecting line 96. Valves 103 and 105 lead to a common manifold which includes a common outlet 102 through which reagents or solvents are passed to reagent/solvent feed lines 30a, 30b, 30c, 30d and 30e.

The vacuum actuated valve assemblies 77-80 are preferably positioned in a portion of the sequence system providing a temperature controlled environment so that they will be maintained at a temperature equal to or greater than the temperature of the reaction cup 21. Preferably, the valve assemblies 77-80 are contained within heated chamber 24, with the length of feed lines 76-96 maintained at a minimum. The vacuum actuated valve assemblies and their maintenance at a selected elevated temperature are important features of the present invention in that they assure condensate will not be

formed in feed lines 30a-30c but rather only in the reaction cup 21 from which vapors originate, eliminating any formation of residue restrictions within the feed lines.

The vacuum actuation of valve assemblies 77-80 is derived from the vacuum pump 32 and is described in detail in reference U.S. Patent No. 3,725,010. The vacuum is applied by way of vacuum line 81 and a plurality of control lines 83a, 83b, 83c, 83d, 83e, 83f, 83g and 83h, 83i, and 83j. An auxiliary vacuum tank 82 is connected to vacuum line 81 to serve as a back-up vacuum source.

To assure that the reagents and solvents are maintained oxygen free, the cessation of vacuum actuation of valves 77-80 is followed by feeding nitrogen through the control lines 83 to vacuum ports 104 of each valve unit 103 and 105. In the case of the valve units 105a-105e associated with the reagent reservoirs R1-R5, the nitrogen is obtained from the four nitrogen feed lines 67a-67e, by means of nitrogen feed lines 85a, 85b, 85c, 85d and 85e, each of which is connected to a connecting line 83 by solenoid actuated valves 89b, 89d, 89f, 8h and 89j, respectively. The nitrogen for valve units 103a-103d associated with the solvent reservoirs S1-S4 is obtained from distribution manifold 60, through four

separate lines 86a, 86b, 86c and 86d by way of four solenoid actuated valves 89a, 89c, 89e and 89g. The solenoid valves 89a-89j interconnect vacuum line 81 or nitrogen feed line 86a-86e and 85a-85e with connecting lines 83 leading to the vacuum port 104 of each valve unit. It should be noted that solvent reservoirs S1-S4 can be provided individual nitrogen carrying lines and related solenoid valves if such is deemed necessary.

Each reagent reservoir R1-R5 is provided a constant pressure uniform delivery system to insure that a repeatable, predetermined volumetric amount of reagent is delivered to the reagent cup 21 throughout each cycle of operation of the protein/peptide sequencer. The delivery system is illustrated in Fig. 2 which depicts one reagent reservoir R4 and its associated delivery system components. The delivery systems for the remaining solvent and reagent reservoirs are identical.

The volume of fluid, solvent or reagent delivered to the reaction cup 21 is a function of the internal diameter of, (or in other words restriction afforded by), each feed line, the particular fluid viscosity and density both of which are a function of temperature, delivery pressure and the length of time the flow control valve remains open. The inner diameter of the feed line is of course for all practical purposes fixed, while the

open time of a flow control valve may be accurately governed by the system control unit. With these system variables controlled the fluid flow rate becomes a function of the temperature of the fluid, i.e., the feed lines 30, and the pressure associated with the delivery system.

The primary function of the delivery system is to deliver constant measured amounts of reagent or solvent from their respective reservoir 58 to the reaction cup 21. It must be highly insensitive to room ambient temperature changes. The delivery system also must provide a constant delivery pressure thereby achieving a constant fluid flow rate which enables the delivery of a repeatable and exactly predetermined amount of solvent/reagent into the reaction cup 21 throughout the degradation process.

As previously described and now with reference to Fig. 2, reagent reservoir R4 is connected to nitrogen feed line 67d which includes a flow control valve 68d, and to vent manifold 72 by way of vent line 95 which is controlled by vent control valve 74d and adjustable metering valve 75. Finally, the reservoir R4 is connected to feed line 30d through line 76d and vacuum actuated valve unit 105d. It is desirable to maintain the reagent pressure from the reservoir somewhere between

100-200 mm of Hg depending on the particular nature (volatility and viscosity) of the reagent. Many of the reagents used throughout the degradation process are highly volatile in nature. As a result, the pressure in the reservoir at the delivery line inlet 94 varies as a function of the reagent vapor pressure inside the reservoir as well as from changes in fluid level within each reservoir. Since vapor pressure is a function of ambient temperature variations, the temperature of the reagent both within the reservoir and in a feed line 30 can have a destabilizing effect if not maintained at a constant value.

To maintain a constant pressure through feed line 30 and at inlet 94 of the connecting line 76, the following steps should be performed. Initially, each of the valves 68, 74 and 105D are closed. Metering valve 75 is adjusted to allow a slow vent flow of approximately 30-50 cc. per minute fluid (vapor) flow from reservoir R4 and vent valve 74 is opened. Each time solenoid valve 74d is opened, reservoir R4 is slowly vented to the atmosphere. After a predetermined time, sixty seconds (empirically determined), the total pressure inside the bottle falls below that of the incoming nitrogen pressure.

At this time solenoid valve 74d may be closed for

short term delivery or left open for long term delivery while valve 68d remains open to pressurize the reagent reservoir with nitrogen gas. The nitrogen is allowed to bubble through the reagent for a certain period of time until a dynamic pressure equilibrium is established, namely, a constant pressure at delivery line inlet 94 located at the entrance of connecting line 76. At this time vacuum operated flow control valve 105d is actuated to permit reagent to flow into the reagent cup 21.

The fluid flow rate of the reagent is subject to temperature variations as it travels through the flow control valve 105d and feed line 30d which can affect fluid flow rate and permit formation of residues blocking the fluid feed line. However, as discussed in connection with Fig. 1, approximately 75% of each feed line 30 and flow control valve 105 are located within the heated chamber 24 (or other temperature controlled environment) which maintains these elements at a predetermined constant temperature promoting a constant flow rate and eliminating residue formation due to temperature change.

The described pressure equilibration procedure is performed each time a reagent or solvent is introduced into the reaction cup 21. The effect of this equilibration procedure is to desensitize the sequencer system to random variations in ambient room temperatures

and changes in temperature environment within the sequencer system itself. The sequencer system does not require specialized environmental control equipment such as air conditioning for the room in which it is placed. The described delivery system insures a repeatable introduction of a select predetermined volume of reagent into the reaction cup 21 for each fluid distribution step in the degradation process.

Furthermore, the delivery system prevents cross contamination between various reagents (and solvents though this is not critical). That is, the solenoid valve 68d located in nitrogen feed line 67d remains closed while vent valve 74 is opened venting the reagent reservoir 58 (R4 in Fig. 2) until the pressure inside the reservoir falls below that of the pressure of nitrogen being applied to the reservoir thereby preventing reversion flow of reagent from the reservoirs into the nitrogen supply line 67. Thus, each reservoir in effect has its own nitrogen supply.

Referring to Fig. 1, a means for removing the excess reagents, reaction products and byproducts from the reaction cup 21 comprises effluent line 151 which enters the reaction cup 21 from above through the support plug 143 and bends toward the interior side wall of the reaction cup 21. The tip of the effluent line 51 is

formed in a manner to provide a pick up which is located in annular groove 101 formed around the upper interior surface of the reaction cup 21 near its top edge. The effluent line 151 leads from the interior groove 101 of the reaction cup 21 to pressure actuated effluent valve 90a which controls fluid flow through line 151. Effluent line 151 continues from valve 90a to selector valve 90b, also pressure actuated which couples effluent line 151 with either a waste bottle 153 or the fraction collector 155, through waste line 154 or collector line 156 respectively depending on the whether the effluent being withdrawn from the sample cup 21 is waste material or sample product for analysis. For example, during the wash stages of the reactants in the sequential degradation process after each coupling step, (e.g., solvent extraction) the excess reagents and reaction byproducts flow to waste bottle 153 via effluent line 151 as controlled by valve 90a which is connected to waste line 154 by valve 90b. Reaction products removed by solvent extraction after a cleavage step are directed to the fraction collector 155 via the effluent line 151, as controlled by valve 90a which is connected to collector line 156 through valve unit 90b which leads to the fraction collector 155.

Both valve units 90a and 90b are pressure actuated (spring return) through connecting lines 157 and 158

respectively which include solenoid actuated valves 161 and 162, respectively, to control the pressurized nitrogen application by way of nitrogen feed line 160.

While fluids, i.e., reagents or solvents, are being introduced into the reaction cup 21 effluent valve 90a is opened and valve 90b is directed to connect line 151 with waste line 154 to interconnect the interior of the reaction cup 21 with waste bottle 153 and into exhaust blower 41. This permits excess vapors to escape from the cup 21 during fluid delivery, preventing a pressure build-up which would effect fluid delivery rate, and reducing vapor entry into the chamber 22 from the interior of cup 21 where reagents/solvents are contained.

A suitable fraction collector 155 includes an array of collection tubes each of which is capable of containing 5 to 10 milliliters of reaction product. The collection tubes are supported by a suitable rack, etc., which is indexable and provided with a suitable automated drive means. The interior of the fraction collector 155 is evacuated by connection with either vacuum pump 32 through vacuum line 32a, solenoid valve 39 and vacuum line 164, or vacuum pump 31 through vacuum line 31a, solenoid valve 39' and vacuum line 164'. Solenoid valve 39 or 39' controlling vacuum application to the fraction collector 155 is operated under direction of the sequencer control system. By evacuating the fraction

collector chamber the final reaction product being collected is immediately dried following their collection in a collection tube to preserve ATZ derivative product of the amino acid for the conversion step and amino acid identification procedures.

The interior of the fraction collector 155 is also connected with an exhaust fan 41 through manual valve 165, solenoid exhaust valve 40, an exhaust line 43 to exhaust reagent vapors from within the fraction collection chamber which may build up during reaction product delivery.

Preferably a fraction collector 155 system is provided, which as shown, permits a conversion reaction step to take place immediately, such as within a collector tube which acts as a second reaction cell, so that the dried reaction product held within the tube/cell is the more stable compound, a PTH derivative of the amino acid, which can then be stored in the collection tube for future analysis for significantly longer periods of time. Such a fraction collector system is fully described in U.S. patent application Serial No. 500,670, and is described in relation to the present automated sequence system as follows. Such a fraction collector 155 comprises a collector rack 135 which contains a plurality of collection tubes 136. The rack is

automatically movable by a drive mechanism (not shown) whereby each tube is advanced to a position under a delivery head 137 to receive an ATZ derivative reaction product to perform the conversion step in the degradation chemistry. The collection tubes are preferably disposable. A heated transport member 138 having a recess designed to engage a lower portion of each collection tube 136 is located below a tube when the tube is positioned below the delivery head 137. The member 138 is temperature controlled by thermoelectric means to provide heat to the tube and its contents when the conversion step reaction is to be performed. Member 138 is vertically movable toward and away from the collector tube and the delivery head 137 to move the collector tube 136 into sealed contact to the delivery head 137. The transport mechanism and temperature control means is more clearly described in patent application Serial No. 500,670 of Ohms et al. As member 138 moves upwardly it encases the collector tube 136 and moves it upward to seal its upper end against the delivery head 137. Once the collection tube 136 is sealed against the delivery head 137 effluent from the reaction cup 21 containing the ATZ derivative product is picked up from the groove 101 and transferred through effluent line 151 and collector line 156 to the delivery head and into the collector tube 136, as directed by valves 90a and 90b. Vacuum line 164

withdraws undesired vapors which may pass into the contained space within the fraction collector.

The delivery head 137 comprises a number of ports which are connected to various feed lines for reagents and gases which are not described here, but which are fully disclosed in the above referenced patent application. Inert gas enters the collector tube through entry ports in the delivery head 137 in a manner to create a swirl within the collector tube 136. This speeds evaporation and enhances mixing of the reactants in the tube with aqueous acid introduced to perform the conversion reaction. Evaporative cooling of the contents within the tube is sensed by a temperature sensor and the cooling effect compensated for by thermoelectric heating through the temperature controlled transport member 138.

After removal of the solvent by inert gas, an aqueous acid is delivered through a port in the delivery head 137 to initiate the conversion chemistry. The tube is heated to heat its contents and commence the conversion reaction. Heating is accomplished through transport member 138. The conversion reaction yields PTH derivative of the amino acid which may be analyzed to identify the amino acid. After a reaction is completed an inert gas is directed into the tube 136 through swirl ports in the delivery head 137 in a swirling manner to

evaporate the contained acids and solvents from the tube through a vent line to the waste bottle 153, to dry the contained PTH reaction product. After the operations for the conversion step are complete the transport member 138 is lowered and the tube containing a dried PTH derivative of the amino acid is placed back in the rack 135. The rack 135 is then advanced to position the next collection tube 136 for communication with the delivery head 137. A more detailed description of the fraction collector providing a system for performing the conversion steps in the degradation reaction is described in above referenced patent application Serial No. 500,670.

Referring to Fig. 1c and the reaction cell and chamber assembly 20, the reaction chamber 22 is provided with a drain port 145 located at a lowermost portion of the chamber 22. The drain port 145 is connected through waste line 147 and solenoid operated valve 146 to the waste bottle 153 for collection of removed vapors, waste materials and wash solution. The drain port 145 provides a means to remove reactant vapors, condensed or residual reactants and wash solution from the reaction chamber 22, which advantageously can be assisted by the flow of nitrogen through the interior of reaction cup 21 and out into reaction chamber 22 to the drain port 145.

Provision of the drain port 145 permits complete

washing of the interior of reaction cup 21 and reaction chamber 22 at the end of each amino acid degradation in the protein/peptide chain. Specifically, as the reaction cell 21 is spun at high speed wash solution can be introduced from a reagent reservoir into the interior of the reaction cup 21 through a feed line 30. The wash solution covers the interior walls of the reaction cup 21, as does solvent or reagent when they are introduced therein. Wash solution is continued to be introduced into the reaction cup 21 until a sufficient volume enters that turbulent action in the solution occurs within the cup and the cup is overflowed with wash solution which exits between the upper edge of the reaction cup 21 and the support plug 143. As the wash solution exits the reaction cup 21 through the gap between its upper edge and the support plug 143 it is thrown off by the centrifugal force generated by the spinning cup and sprayed against the interior wall of the reaction chamber 22 permitting wash solution to wash down the interior of the reaction chamber 22. Sufficient space is provided below the reaction cup 21 and the floor of the reaction chamber 22 to permit wash solution to flow to the drain port 105 for removal.

Following overflow of wash solution from the reaction cup 21, introduction of wash solution is ceased and effluent line 151 is connected through valve 90a amd

90b to waste line 153 leading to the waste bottle 153. The reaction cup 21 continues to spin as wash solution is removed from the interior of the cup through effluent line 151 by way of the pick up in the groove 101 of the reaction cup 21. Nitrogen valve 56 is then opened to introduce pressurized nitrogen into the interior of the cup 21 to assist in removal of wash solution and subsequently to dry the interior of the reaction cup 21 and the reaction chamber 22. Following completion of cup and chamber drying, waste line 147 and effluent line 151 are closed by their respective valves 146 and 90a, and the reaction cup 21 and reaction chamber 22 are left in a cleaned condition for performance of the next peptide/protein degradation.

This method of washing the reaction cup 21 and chamber 22, which includes introduction of wash solution and inert gas into the interior of the reaction cup 21, "spraying" of wash solution from the cup 21 onto interior portions of the reaction chamber 22, and removal of the wash solution and inert gas through a drain port 145 at the lowermost portion of the reaction chamber 22 (and through effluent line 151) provides a unidirectional flow of solvents, reagents and solution into the reaction cup 21 and out of the chamber 22. The unidirectional flow of fluids through the reaction cup 21 and chamber 22 is an important feature of the present invention in that

residual materials, condensates, reaction byproducts and other fluids which are removed from the reaction cup 21 cannot be reintroduced through reversion flow into the reaction cup 21 to contaminate subsequent reactions in the degradation process. In other words, since all wash cycles of the reaction cup 21 and chamber 22 are performed utilizing a unidirectional flow out of the reaction cup 21 it is assured that all residual materials and reaction byproducts are removed from the reaction chamber 22 in a manner that assures they cannot contaminate subsequent reactions.

This is also true during vacuum purging and drying operations within the reaction chamber 22 which are generally assisted by the flow of nitrogen into the reaction cup 21 and out through vacuum port 108. The removal of waste materials and sample reactant drying may be accomplished by a combination of vacuum application to the reaction chamber 22 and the introduction of positive pressure inert gas flowing through the reaction cup 21 and the reaction chamber 22 and out through the drain port 145 and/or vacuum port 108. The unidirectional flow through the reaction cup 21 and reaction chamber 22 is enhanced by the flow of nitrogen. Nitrogen is introduced into the reaction cup 21 by nitrogen feed line 46 and flow is directed out between the upper edge of the reaction cup 21 and support plug 143 and into the

reaction chamber 22 to the drain port 145. The enhanced unidirectional flow as provided by the pressurized nitrogen, is an important feature of the present invention.

A temperature control means is provided to maintain the reaction chamber 22 and the reaction cup 21, cup drive 29, reagent/solvent flow control valves 77-80 and related feed lines 30, vacuum control valves 36, 38, 38', nitrogen control valve 56, effluent line control valves 90a and 90b and drain control valve 106, at a constant stable temperature.

Temperature control is provided by air circulation throughout the heated chamber 24 and any additional associated temperature controlled chambers. For example, air circulation is provided to the chamber 24 by a fan-heating element 27 which is mounted in the base plate 26. Air is driven by the fan through the comprised heating element which is thermostatically controlled to direct air circulation throughout the heated chamber 24 to maintain the chamber and contained system components at the desired constant temperature. As discussed earlier, the heated chamber 24 is comprised of a suitable insulated material to maintain temperature stability and obtain efficiency. In operation the fan-heating element 27 draws air from a lower portion of the chamber 24,

forces air through the heater element and upwardly into the upper portion of the chamber 24 and over the reaction cup and chamber assembly 20. The heated air leaving the fan-heating element 27 circulates through the upper portion of chamber 24 and downwardly into the lower portion of the chamber containing the cup drive 29 and other valve elements through defined openings in the base plate 26. The heated chamber 24 and its internal surfaces as well as all elements disposed within the reaction chamber, are thus maintained at a substantially uniform temperature.

The reaction chamber 27, reaction cup 21, and cup drive 29 are located centrally within the heated chamber 24 so that they will be maintained at a substantially uniform predetermined and constant temperature, preferably around 50° C. These elements have the greatest greatest mass and largest heat sink area and thus are the slowest to respond to changes in temperature of the distributed air. This means that under proper temperature control the interior of the reaction cup 21 will tend to be at the lowest temperature to keep condensates highly volatile reagents and buffers forming within the reaction cup 21 and and not within the reaction chamber and associated fluid elements, e.g., delivery lines and flow control valves. It should be noted that it is important to maintain the reaction cup

21 at a temperature level at or slightly lower than the other components contained within the heated chamber 24 to insure that the condensate from volatile reagent/solvent vapors will be formed within the interior of the reaction cup thereby assuring that vapors from the volatile liquids will be retained in the cup.

A second temperature control system (not shown) may be provided to maintain a constant predetermined temperature environment within the fraction collector 155.

Temperature within the reaction cup 21 and within the reaction cup 21 is continually monitored by a temperature probe such as a thermistor and preferably a plurality of temperature probes. Another temperature probe is positioned near the fan-heating element outlet to continuously sense the temperature of the air emerging therefrom. The temperature probes are electrically connected to the sequencer control means to determine operation of the fan-heating element 27. The fan-heating element is controlled by the sequencer control means to overheat the heated chamber 24 during start-up periods and to react thermostatically following obtaining temperature equilibrium within the heated chamber 24 to maintain the temperature at a selected level, previously stated as being preferably 50° C.

The over-all operation of the presented protein/peptide sequencer is provided by a programmable control means which is designed to control the temperature profile, delivery of sample, solvents and reagents, reagent cup rotation delivery of inert gas and application of vacuum, positioning of the tubes at the fraction collector and all other operations of the sequencer system. This control means generally includes a microprocessor or microprocessor based computer of many known types and designs which are capable of controlling the functions described herein for an automated peptide/protein sequencer.

For purposes of illustration the operation of the presented automated protein/peptide sequencer will be discussed in relation to the Edman process, the phenylisothiocyanate degradation reaction devised by Edman, et al. discussed supra. However, it should be apparent that the present automated system may successfully carry out many degradation, sequencing or synthesis processes by merely varying the instructional (computer) program driving the control means accordingly.

Generally speaking the phenylisothiocyanate degradation reaction process consists of the formation of a phenylthiocarbamyl (PTC) derivative of the protein or

peptide sample being investigated and the cleaving of the N-terminal PTC amino acid derivative as a thiazolinone derivative. Basically, as described the process involves three distinct steps or reactions commonly referred to as (1) the coupling reaction step, (2) the cleavage reaction step and (3) the conversion reaction step. In the coupling step the protein or peptide sample is first exposed to a coupling medium consisting of PITC and an alkaline buffer to form the PTC derivative of the terminal amino acid of the peptide/protein. After completion of this coupling reaction excess reagents and byproducts are washed away by solvent extraction and residual extracting solvent is evaporated.

Separation of the PTC derivative from the peptide/protein is performed in the cleavage reaction step yielding an ATZ derivative of the amino acid. In the cleavage step the anilino-thiazolinone (ATZ) derivative is cleaved from the peptide/protein chain by exposing the PTC derivative to anhydrous acid in an oxygen free environment which cleaves the PTC derivative and converts it to an ATZ derivative. The ATZ derivative is extracted by a suitable organic solvent and transported to another reaction cell for the next step. The protein or peptide sample remaining in the reaction cup is then dried by a combination of nitrogen circulation and vacuum application to prepare it for

succeeding cycles of successive degradation of the amino acids comprising the molecular chain structure.

The conversion reaction converts the 2-anilino-5-thiazolonone (ATZ) derivative which is highly unstable to the isomeric 3-phenyl-2-thiohydantoin derivative (PTH) since it has been found that the extracted thiazolinone derivatives are much too unstable to render them suitable for storage prior to identification processes. In fact, it has been found that an automated conversion reaction system such as provided by the described fraction collector which comprises a second reaction vessel for immediately carrying out the conversion reaction step and storing the produced stable PTH derivative in a dried form is highly advantageous and desirable. Typically, after the conversion reaction step the sample residues (amino acids) are identified by chromatography using apparatus of the type manufactured by Beckman Instruments, Inc. identified as a Model 344M High Pressure Liquid Chromatography System.

Operation of the peptide/protein sequencer begins with the placing of a sample in solution with solvent in the sample injection valve 200. The sample is deposited into the reaction cup 21 while spinning. The solution assumes the form of a thin film on the inner wall of the reaction cup as a result of the centrifugal force field

generated by spinning the cup. Solvent in the thin sample film evaporates and is removed as a vapor by the flow of inert gas (nitrogen) into the interior of reaction cup 21 through the nitrogen feed line 46 and out through effluent line 151 and/or waste line 147 and vacuum line 31a. Nitrogen gas entering the cup 21 and chamber 22 is preferably preheated, by including a substantial length of nitrogen feed line 46 within the temperature controlled chamber 24 (or by a preheater), before it enters the reaction cup 21 enhancing vaporization of the solvents therein and assisting constant temperature maintenance within the reaction chamber 22. The inert gas preferably enters the bottom of the reagent cup 21 and flows over the thin film held against the interior wall of the spinning cup to extract the solvent vapor. The gas is exited through effluent line 151, and/or vacuum port 108 communicating with the reaction chamber 22.

Next, PITC or other suitable coupling agent is added in solvent solution (such as heptane) into the reaction cup 21 where it forms a thin film over the dried sample film on the inner wall of the reaction cup 21. A major portion of the solvent is again removed by a flow of inert gas into the cup 21 and over the thin film, such gas and vapor being removed to waste receptacle 153 as

described above. The PITC remains as a thin film over the sample film to interact in the coupling reaction.

A buffer of suitable pH and composition for maintaining the desired 9.5 pH of the coupling reactants is delivered into the reaction cup 21. It should be noted that since the sample injection valve 200 is included in the buffer feed line, introduction of buffer through this feed line "washes" any sample remaining in the line into the reaction cup 21. The waste line 101 or 154 via effluent line 151 is opened via valves 90a and 90b to the waste receptacle 153 to prevent pressure build-up during buffer solution delivery. The reaction chamber is then isolated and the coupling reaction is allowed to take place in an oxygen free environment within the reaction cup.

When the coupling reaction is completed the volatile portion of the reactants is removed by vacuum applied to the reaction chamber and/or with a flow of inert gas (nitrogen through feed line 46) through the reaction cup 21. Vacuum and nitrogen flow continues until the reaction product is semi-dry. The semi-dried film on the inner wall of the reaction cup 21 is then washed with ethyl acetate and butyl chloride or other suitable solvents to extract excess coupling reagent and reaction byproducts. These materials are removed through

the effluent line 151 assisted by the positive pressure inert gas (nitrogen). The thin film containing the phenylthiocarbamyl (PTC) derivative is now dried by the flow of inert gas, i.e., nitrogen, through the reaction cup 21.

As operation of the sequencer continues, anhydrous acid for performing the cleavage step is delivered into the reaction cup 21 and is flowed by centrifugal force over the thin film consisting of the coupling reaction product on the inner wall of the reaction cup. The acid cleaves a residue of the PTC derivative as an anilino-thiazolinone (ATZ) derivative. The cleavage acid vapor is removed by vacuum and inert (nitrogen) gas flow through the reaction cup 21 as has been described. Solvent is then delivered to the reaction cup 21 to extract the cleaved ATZ derivative product from the reactants. The solvent carries the cleaved ATZ derivative to the fraction collector 155 through effluent line 151 and collector line 156.

The ATZ reaction product extracted from the reaction cup 21 is delivered to a collector tube within the fraction collector. If the fraction collector is designed to permit occurrence of a conversion reaction, the ATZ derivative is first dried and then aqueous acid is introduced into the collector tube which becomes a

second reaction cell to generate the conversation reaction. After acid introduction inert gas is swirled within the tube to mix the reactants and dry the reaction product which consists of a PTH derivative of the amino acid. The tube is then removed for storage until amino acid identification processes are to be performed.

The automated protein/peptide sequencer described herein uses the following reagents and solvents in operation:

Reagent 1 — 2.5% solution of phenylisothiocyanate (PITC) in heptane.

Reagent R2 — 0.1 molar Quadrol, a nonvolatile base utilized as a reaction buffer in a 50/50 mixture of propynol and water with pH adjusted to 9.5 by trifluoroacetic acid. (Quadrol is a registered trademark of the Wyandotte Chemical Corporation and is generically described as N,N,N',N',-tetrakis-[2-hydroxypropyl]-ethylene diamine)

Reagent 3 — anhydrous N-heptafluorobutyric acid.

Reagent 4	Wash solution of 50-50 propanol and deionized water.
Reagent 5	25% solution of trifluoroacetic (TCA) acid with deionized water.
Solvent 1	Used to pass inert gas (nitrogen) to purge feed line.
Solvent 2	Ethyl acetate.
Solvent 3	Used to pass inert gas (nitrogen) to purge feed line.
Solvent 4	Butyl chloride w/ 1.0 mg. DTT in 1.0 cc of acetonitrile..

For optimum performance it is desirable to purify the reagents and solvents to remove all oxidizing agents and all chemical entities that react with the free amino groups of a peptide or protein, co prevent blocking the protein/peptide chain from further degradation. This is particularly critical with aldehydes since they tend to react with the N-terminal amino acid group resulting in a progressive decrease in yield from the degradation reaction. Purification may be accomplished by any one of the ways known in the chemical arts.

An understanding of the operation of the presented automatic protein/peptide sequencer can be obtained from a discussion of the steps which the instrument performs in one cycle of the degradation of a peptide or protein to derive a single amino acid identifier. Each cycle of degradation derives one amino acid unit. Thus, a protein containing N amino acid units requires N cycles of degradation to successfully determine the amino acid sequence characterizing a protein or peptide. For complete understanding, each step which the sequencer system performs will be described following, with a discussion of the system components affected referring to Figs. 1A-1D.

Initially, valves 48 and 48A which control flow of nitrogen from the nitrogen storage bottles 44 and 45 are opened to permit nitrogen to enter the nitrogen feed lines of the sequencer system. A select number of collector tubes for receiving reaction product (ATZ derivative of the amino acid) are placed in the supporting rack of the fraction collector 155. Hand operated valves 57, and 69 and solenoid valves 66 and 74 are opened flowing pressurized nitrogen through the reagent solvent reservoirs. Metering valves 75 are opened permitting nitrogen to bubble through each of the reservoirs 58 to purge the reservoirs and related

nitrogen feed and vent lines of oxygen and air, etc. After a few minutes, each of five metering valves 75 is adjusted until a slow vent of approximately 30-50 cc. per minute flow is obtained. The control means, cup drive 29, vacuum pumps 31 and 32, fan heating element 27, exhaust fan 41, fraction collector drives and other operative elements of the sequencer are turned on. Once the heated chamber 24 and its contained system components are stabilized the desired temperature operation of the sequencer commences.

Step 1: Polybreen is deposited in the reaction cup 21.

Polybreen is introduced into the reaction cup 21 through use of a syringe, pipette or other suitable device, by inserting the syringe into the receiving port 201 of the sample valve 200 and depositing the polybreen therein.

The sample valve is set to open the sample receiving port to buffer feed line 30b, which generally flows the Quadrol buffer solution from the reagent reservoir R2 Cup drive 29 is controlled to spin reaction cup 21 at slow speed (1200 rpm). With all other valves controlling fluid communication with the extractor chamber 22 closed, vacuum valve 38' is opened permitting

restricted vacuum to be applied to the interior of the reaction cup 21. The applied vacuum slowly pulls the Polybreen from the receiving port of the sample valve through feed line 30b into the spinning reaction cup. As the Polybreen enters the cup 21, the centrifugal force generated by the spinning cup flows the Polybreen along the bottom and side wall of the reaction cup in the form of a thin film.

Polybreen is a highly adhesive copolymer which when applied to the surface of the reaction cup provides a strong and stable support for a protein or peptide undergoing degradation through the reactions herein described.

Step 2: The Polybreen film in the spinning reaction cup is dried.

Valve 38 is permitted to remain open for a short period of time to initiate drying of the Polybreen in the reaction cup. Shortly, valve 36 is opened applying full vacuum to the reaction chamber 22 through drain port 108 and thus to the interior of the reaction cup 21. Nitrogen valve 56 is opened, as the sample valve 143 is closed, permitting nitrogen to flow through nitrogen feed line 46 into the center of the interior of the reaction cup, outwardly and over the walls of the

interior of the cup, out through the space between the cup and support plug 143, into the reaction chamber 22, and out through the vacuum port 108 adjacent to drain port 145. The nitrogen and extracted vapors are removed through vacuum line 31a through exhaust fan 41. Nitrogen flow through the interior of reaction cup 21 and reaction chamber 22 is ceased as the Polybreen reaches a dry state. This is accomplished by closing valves 36 and 56 in order.

Step 3: Polybreen in the reaction cup is washed.

Valves 90a and 90b are directed through application of vacuum through lines 157, 158 to connect effluent line 151 with waste line 154 leading to the waste bottle 153.Slight pressure within the reaction chamber 22 from delayed closing of pressurized nitrogen feed line 46 initiates flow through effluent line 151. Cup drive 29 is directed to spin the reaction cup 21 at high speed (1800 rpm) to generate increased centrifugal force to drive the wash solvent to the effluent line 151 for collection. Valve 79 controlling the flow of solvent S2 (ethyl acetate) is directed through application of vacuum (and pressurized nitrogen) through respective lines 83c and 83d as controlled by valves 89c and 89c to feed solvent S2 into the interior of the reaction cup 21 through feed line 30B. As the solvent flows through line

30B any Polybreen remaining within the feed line is washed into the reaction cup 21. The solvent entering the reaction cup is directed as a film over the Polybreen film contained therein, by high centrifugal force generated by rotating cup 21 at high speed. Solvent S2 is collected from the groove 101 as the high centrifugal force drives the continuing flow of solvent outwardly and upwardly over the Polybreen film.

Solvent entering the groove is collected through the pick up of effluent line 151 and removed through valve 90a and 90b to the waste container 153. Delivery of the solvent S2 from its reservoir is accomplished as later described.

Polybreen film is again dried. Flow control valve 56 is opened permitting pressurized nitrogen to enter the interior of the cup 21 forcing solvent from the interior of the cup out through effluent line 151. While pressurized nitrogen is continued to be applied valve 38 is permitted to open to initiate drying the Polybreen film of the remaining solvent. After a short period vacuum valve 36 is opened, applying full vacuum and flowing nitrogen through the interior of the reaction cup 21 as described in Step 2 to completely dry the Polybreen film.

Step 4: Polybreen film is subject to all chemicals and steps of a degradation cycle.

All the steps of a degradation cycle of the Edman process as performed and hereinafter described for the present sequencer system, are performed on the Polybreen film contained within the reaction cup.

Step 5: Sample is added to the reaction cup

Sample in a solvent solution is introduced into the reaction cup 21 similarly to the introduction of the Polybreen, through insertion of a syringe containing the sample material into the receiving port 201 of the sample valve 200 and depositing the sample therein. The sample valve is set to open the sample receiving port to buffer feed line 30b, which generally flows the Quadrol buffer solution from the reagent reservoir R2. Cup drive 29 is controlled to spin reaction cup 21 at slow speed (1200 rpm). With all other valves controlling fluid communication with the reaction chamber 22 closed, vacuum valve 38 is opened permitting restricted vacuum to be applied to the interior of the reaction cup 21. The applied vacuum slowly pulls the sample from the receiving part of the sample valve 200 through feed line 305 into the spinning reaction cup. As the sample solution enters the cup 21, centrifugal force generated by the spinning

cup flows the sample along the bottom and side wall of the cup over the Polybreen film in the form of a second thin film. The adhesive characteristics of the Polybreen copolymer adheres the sample to the bottom and interior wall of the reaction cup 21 providing a strong and stable support for the protein/peptide undergoing degradation. Vacuum valve 38 is closed once sample has been fully introduced into the cup 21.

Step 6: Pressure in the reaction chamber 22 is equalized.

Cup drive 29 is directed to spin reaction cup 21 at its highest speed (1800 rpm). Under the combined influence of the centrifugal and gravitational force fields existing within the interior of the reaction cup 21, the sample migrates towards the wall of the reaction cup and flows over the Polybreen film supported thereon becoming attracted thereto. Valve 56 is opened to admit pressurized nitrogen gas into the reaction cup 21 which nitrogen serves to counteract the withheld vacuum and pressurize the reaction chamber 22 to minimize the pressure differential between the reaction chamber 22 and the reservoirs 58. This is done to prevent a high preliminary surge in delivery of reagents or solvents to the reaction cup 21 resulting in erroneous fluid quantities being supplied. (Valve 39' may be opened to

connect the vacuum pump 31 with the fraction collector 155 to apply a vacuum to the interior of the fraction collector to assist the drying of the reaction product residues contained in collector tubes therein which have been previously collected).

Step 7: The reservoir for reagent R1 (PITC) is vented.

Valve 74c is opened to slowly vent reservoir R1 to the atmosphere to drop the total pressure inside the reservoir below that of the pressurized nitrogen to be applied.

Step 8: Reagent reservoir R1 (PITC) is pressurized.

Valve 68a is opened permitting nitrogen to flow through line 67a into reagent reservoir R1 establishing a dynamic equilibrium pressure condition within the reservoir, as previously described with reference to Fig. 2.

Step 9: Reagent R1 (PITC) is delivered into the reaction cup 21.

Cup drive 29 is controlled to spin the reaction cup 21 at high speed (1800 rpm) or low speed (1200). Valves 68a and 74c remain open so that nitrogen continues to flow through the reservoir R1. Valve 56 remains open permitting nitrogen to flow into the reaction cup 21. Valve 89b is actuated applying vacuum to the connecting line 83b to apply vacuum to valve unit 105a opening the valve and allowing the reagent to flow from the reservoir R1 through feed line 30a to the reaction cup 21. Valve 106 is opened to prevent an excessive pressure build up in the reaction chamber 22 while reagent is being delivered, permitting vapor in the reaction chamber 22 to escape to the atmosphere via line 107 to the waste container 153 and on to the exhaust fan 41.

Reagent entering the cup flows over the thin sample film held on the bottom and interior walls of the reaction cup 21 due to the centrifugal force generated by the spinning cup. Reagent delivery is ceased by actuating Valve 89b to connect line 83B with connecting line 85A permitting nitrogen to break the vacuum and close valve unit 105A shutting off the flow of reagent R1 from its reservoir to the reaction cup 21, as the reagent film reaches a preselected level along the side of the reaction cup 21. Valves 68a and 74c are closed ceasing nitrogen flow through reservoir R1. Valve 106 is closed.

Step 10: Blow through reagent R1 feed line.

Valve 66a is opened pressurizing the empty solven reservoir 57. Valve 89a is actuated applying vacuum to connecting line 83a to apply vacuum to valve unit 103a opening the valve allowing pressurized nitrogen to flow through feed line 30a to blow all remaining reagent into the reaction cup 21. After a moment, valve 89a is reversed to connect line 83a with nitrogen line 86a permitting pressurized nitrogen to break the vacuum applied to valve unit 103a and close the valve ceasing flow of nitrogen through feed line 30a. Valve 66a is closed. Valve 106 remains open throughout this operation to prevent excessive pressure build up in the reaction chamber 22.

Step 11: Restricted vacuum is applied to the reaction cup 21 and reaction chamber 22.

Valve 56 is closed, shutting off nitrogen flow to the reaction chamber 22 through nitrogen feed line 46. Valve 38 is opened connecting vacuum pump 31 through the restricted vacuum line 110 to apply vacuum to the reaction cup 21 and the reaction chamber 22 to evaporate a portion of the heptane carrier solvent which is used as a vehicle for the PITC reagent R1.

Step 12: Pressure in the reaction chamber 22 is equalized.

Valve 38 is closed ceasing vacuum application to the reaction cup and chamber. (Valve 39' may be opened connecting the vacuum pump 31 with the interior of the fraction collector 155.) Valve 56 is opened supplying pressurized nitrogen to the reaction cup 21 and the reaction chamber 22 for the purposes outlined in Step 2, i.e., pressure equalization.

Step 13: Reagent reservoir R2 (buffer) is vented.

Valve 74' is opened to slowly vent reservoir R2 to the atmosphere to drop the total pressure inside reservoir R2 below that of the pressurized nitrogen to be applied.

Step 14: Reagent reservoir R2 (buffer) is pressurized.

Valve 68b is opened permitting nitrogen to flow through line 67b into the reagent reservoir R2 establishing a dynamic equilibrium pressure condition inside the reservoir R2, as previously described with reference to Fig. 2.

Step 15: Reagent R2 (buffer) is delivered to the reaction cup 21.

Cup drive 29 is controlled to spin the reaction cup 21 at low speed 1200 rpm). Valves 68b and 74i remain open so that nitrogen continues to flow through the reservoir for reagent R1. Valve 56 remains opened permitting nitrogen to flow into the reaction cup 21. Valve 89d is actuated to apply vacuum to line 83d opening valve unit 105b and allowing reagent to flow from reservoir R2 through feed line 30b into the reaction cup 21. Reagent R2 is the buffer Quadrol and as the reagent flows through the feed line 30b it washes any remaining sample in the feed line 306 into the reaction cup 21. Valve 106 is retained open to prevent an excessive pressure build up in the reaction chamber 22 while the reagent is being delivered, permitting vapor in the reaction chamber 22 to escape to the atmosphere via line 101 to the waste container 153 and on to the exhaust fan 41.

Reagent R2 entering the cup flows over the thin sample film held on the bottom and interior walls of the reaction cup 21 and mixes with the thin film of reagent R1, due to the centrifugal force generated by the spinning cup 21. Reagent delivery is ceased by actuating valve 89d to connect line 83d with connecting

line 85b permitting nitrogen to break the vacuum and close valve unit 105b shutting off the flow of reagent R2 from its reservoir to the reaction cup 21 when a preselected volume of reagent R2 is introduced.

Valves 68b and 74i are closed shutting off the nitrogen flow through reservoir R2. (Note: Reagent R2 feed line may be 50% emptied of buffer solution by beginning delivery of solvent S2 through the feed line 30b.)

At this point it should be noted and emphasized that both reagents R1 and R2 are introduced into the reaction cup 21 while the reaction cell is spinning at high speed (approximately 1800 rpm). The combined volume of R1 and R2 is controlled to reach under these conditions a level of about three quarters above the height of the bottom of the cup. This insures that the reagents completely cover the peptide/protein sample film on the interior wall of the cup to prevent a portion of the sample being unreacted or partially reacted, such as a ring of unreacted sample remaining and accumulating about the inner surface of the reaction cup 21.

Step 16: The coupling reaction step is permitted to occur (5 to 20 minutes).

Valve 56 is closed shutting off the nitrogen flow to the reaction cup 21 and chamber 22 permitting the coupling reaction to proceed in an inert gas atmosphere.

Step 17: Coupling reaction step continues.

Cup drive 29 is controlled to reduce the speed of the cup 21 to 1200 rpm pulling the sample reagent down toward the bottom of the reaction cup 21. The coupling reaction continues to fully involve the sample and form the phenylthiocarbamyl (PTC) derivative.

Step 18: Restricted vacuum is applied to the reaction cup 21 and chamber 22.

While the reaction vessel 21 is spinning at low speed (1200 rpm), valve 38 is open providing a restricted vacuum application through line 111 to the reaction cup 21, and thus chamber 22, to initiate drying of the constituents in the reaction cup 21.

Step 19: Full vacuum is applied to the reaction chamber to dry reaction product

Vacuum Valves 38 and 36 are opened in order to apply full vacuum to the reaction chamber 22 via vacuum line 109. Nitrogen valve 56 is then opened to

supply nitrogen flow through the reaction cup 21 and outwardly into the reaction chamber 22 to continue the drying of the reaction product.

Step 20: Pressure in the reaction chamber 22 is equalized.

Valves 36 and 38 are closed ceasing vacuum applications to the reaction chamber 22. (Valve 39 may be opened again applying vacuum to the fraction collector 155.) Valve 56 is allowed to remain open to feed nitrogen into the reaction chamber 22 which nitrogen pressure serves to counteract the vacuum in the reaction chamber and pressurize the chamber to minimize the pressure differential between the reaction chamber 22 and reservoirs 58, as described in Step 5.

Step 21: Valve 56 is closed shutting off the pressurized nitrogen flow to the reaction chamber 22.

Step 22: Solvent reservoir S2 (ethyl acetate) is vented.

Valve 74f is opened to slowly vent solvent reservoir S2 to the atmosphere to drop the total pressure

inside the reservoir below that of the pressurized nitrogen to be applied.

Step 23: Solvent reservoir S2 (ethyl acetate) is pressurized.

Valve 66b is opened permitting nitrogen to flow through line 59, distribution manifold 60 and line 62 into the solvent reservoir S2 to establish a dynamic equilibrium pressure condition inside the reservoir S2, as previously described with reference to Fig. 2.

Step 24: Solvent S2 (ethyl acetate) is delivered into the reaction cup 21.

Valves 66b and 74f remain open. Valve 56 is opened permitting nitrogen to flow into the reaction cup 21 and chamber 22. Valve 162 is opened to couple valve unit 90A of valve 152 to applied vacuum interconnecting effluent feed line 151 with waste bottle 153. Valve 89c is actuated to apply vacuum through connecting line 83c to open valve unit 103b to permit solvent S2 to flow from reservoir S2 through feed line 30b to the reaction cup 21. Solvent S1 is introduced into the reaction cup and allowed to flow over the reactants as a film over the wall of the reaction cup and upwardly to the groove 101. The reagent S2 extracts reagents and byproducts

from the reactants and is scooped by the pick up of effluent line 151 in the groove 101 and directed therethrough carrying the extracted reagents and byproducts into the waste bottle 153. Solvent delivery is ceased by actuating valve 89c to connect line 83c with connecting line 86b permitting nitrogen to break the vacuum and close valve unit 103b shutting off the flow of solvent S2 from its reservoir to the cup 21. Valves 66a and 74f are closed shutting off flow of nitrogen through the solvent reservoir S2.

Step 25: Pause.

Valves 56 and 90A remain open and valve 106 is opened to permit nitrogen to circulate through the reaction cup 21 and chamber 22 to assist in the removal of the remaining solvent through evaporation.

Step 26: Restricted vacuum is applied to the reaction cup 21 to dry the reactants.

Valve 38 is opened providing a restricted vacuum application to the reaction cup 21 through line 111 to dry the reactants within the cup.

Step 27: Repeat solvent S2 (ethyl acetate) delivery.

The preceding two steps of delivery of solvent S2 Step 24 and drying of the washed reactants Step 26 are repeated five times.

Step 28: Solvent reservoir S4 (butyl chloride) is vented.

Valve 74h is opened to slowly vent reservoir S4 to the atmosphere to drop total pressure inside the reservoir below that of the pressurized nitrogen to be applied.

Step 29: Solvent reservoir S4 (butyl chloride) is pressurized.

Valve 66d is opened permitting nitrogen to flow through lines 59 and 64 into the reservoir of solvent S4 establishing a dynamic equilibrium pressure condition within the reservoir S4, as previously described.

Step 30: Solvent S4 (butyl chloride) is delivered.

Valves 66b and 74h remain open so that nitrogen continues to flow into the solvent reservoir S4. Valve 56 remains open permitting nitrogen to flow into the reaction cup 21 and chamber 22. Valve 162 is

opened to actuate valve unit 90A connecting effluent line 151 to the waste bottle 153. Valve 89g is actuated to apply vacuum through connecting line 83g open valve unit 103d permitting solvent to flow from the reservoir S4 through feed line 30d to the reaction cup 21. Solvent S4, like solvent S2, washes the reactants within the reaction cup 21 to extract excess reagents and byproducts. The extraction solvent is withdrawn from the reaction cell as it rises up the side of the interior of the reaction cup 21 by way of effluent line 151 as it is scooped from the groove by the pick up and is transferred to waste bottle 153. Solvent delivery is ceased by actuating valve 89g to connect line 83g with connecting line 86d permitting nitrogen to break the vacuum and close valve unit 103d shutting off the flow of solvent S4 from its reservoir to cup 21. Valves 66b and 74h are closed shutting off nitrogen flow through the reservoir S2.

Step 31: Pause.

Valves 56 and 90A remain open and valve 106 is opened to permit nitrogen to circulate through the reaction cup 21 and chamber 22 to assist in the renoval of the remaining solvent through evaporation.

Step 32: Restricted vacuum is applied to the reaction

cup 21 to initiate drying of the reactants.

Valve 162 is closed closing valve 90a and disconnecting effluent line 151 from waste line 154 leading to the waste bottle 153. (Valve 39' is closed if it is used disconnecting the fraction collector 155 from the vacuum pump 31 ceasing vacuum application.) Valve 38 is opened applying a restricted vacuum through line 111 to the interior of the reaction cup 21 while nitrogen is continued to be supplied through nitrogen feed line 46 to the interior of the cup 21 to initiate drying of the reactants in the reaction cup 21, primarily to evacuate the reagents and solvents trapped within the delivery lines 30A and 30B.

Step 33: Pause.

Valve 38 is closed ceasing application of restricted vacuum to the reaction cup and chamber 22. Valve 56 continues to remain open to admit nitrogen into the reaction chamber 22 and the reaction cup 21 which nitrogen serves to counteract the vacuum and pressurize the reaction chamber with clean nitrogen to minimize the pressure differential between the reaction chamber 22 and the reservoirs 58 for reasons previously specified in Step 5. (Valve 39 may be opened to apply vacuum to the fraction collector 155 to assist in drying of the

residues of previously collected sample derivatives.)

Step 34: The reaction product is dried.

The cup drive 29 is controlled to spin the reaction cup 21 at high speed (1800 rpm). Valve 162 is opened actuating valve 90a to interconnect effluent line 151 with the waste line 154 leading to waste bottle 153. As the reaction cup 21 spins nitrogen circulates through the cup and out through the effluent line 151 evaporating the remaining solvents and reagents with the coupled sample reaction product precipitating as a dry film on the wall of the reaction vessel.

Step 35: Application of restricted vacuum to the reaction cup 21.

Valve 162 is closed closing valve 90a to disconnect the effluent line 151 from the waste bottle 153. Vacuum valve 38 is opened in order to apply full vacuum to the reaction chamber 22 via vacuum line 110, 109 to dry the remaining reaction product while nitrogen continues to be introduced through the reaction cup 21 and reaction chamber 22 to assist drying.

Step 36: Vacuum is applied to the chamber 22.

Valve 36 is opened applying full vacuum to the chamber 22 while valve 38 remains open. This achieves optimum drying of the reaction product.

Step 37: Pause.

The cup drive 29 is directed to reduce cup speed to 1200 rpm. Valve 36 and 38 are closed ceasing application of vacuum to the reaction cup 21 and chamber 22. Valve 56 is permitted to remain open to supply pressurized nitrogen to the reaction cup and chamber 22 for pressure equilibrium purposes outlined in Step 5.

Step 38: Reagent reservoir R3 (anhydrous acid) is vented.

Valve 56 is then closed shutting off the nitrogen flow to reaction chamber 22.

Valve 74a is opened to slowly vent reservoir R3 to the atmosphere to drop the total pressure inside the reservoir R3 below that of the pressurized incoming nitrogen to be applied.

Step 39: Reagent reservoir R3 (anhydrous acid) is pressurized.

Valve 68c is opened permitting nitrogen flow through vacuum line 67c into reagent reservoir R3 to establish a dynamic equilibrium pressure condition within the reservoir R3 as previously described with reference to Fig. 2.

Step 40: Reagent R3 is delivered into the reaction cup 21.

Cup drive 29 is controlled to spin the reaction cup 21 at high speed (1800 rpm). Valves 68c and 74a remain open so that the nitrogen continues to flow into reservoir R3. Valve 56 is opened permitting pressurized nitrogen to flow through the reaction cup 21 and chamber 22. Valve 89f is actuated applying vacuum through connecting line 83f to open valve unit 105C permitting reagent R3 to flow from the reservoir for R3 through feed line 30c into reaction cup 21. Valve 106 is opened to prevent an excessive pressure build up in the reaction chamber 22 while reagent is being delivered, permitting vapor in the reaction chamber 22 to escape to the atmosphere via line 107 to the waste container 153.

Reagent entering the cup flows over the dried reaction product held within the cup 21, as described. Reagent delivery is ceased by actuating valve 89f to connect line 83f with connecting line 86c permitting

nitrogen to break the vacuum and close valve unit 105c shutting off the flow of reagent R3 from its reservoir to the reaction cup 21, as the reagent reaches a preselected level along the side of the cup 21. Valves 68c and 74c are closed shutting off nitrogen flow to the reagent reservoir R3.

Step 41: Blow through reagent R3 feed line.

Valve 66c is opened pressurizing the empty solvent reservoir S3. Valve 89c is actuated applying vacuum to connecting line 83c to apply vacuum to valve unit 103c opening the valve allowing pressurized nitrogen to flow through feed line 30c to blow all remaining reagent into the reaction cup 21. After a moment, valve 89e is reversed to connect line 83c with nitrogen line 86c permitting pressurized nitrogen to break the vacuum applied to valve unit 103c and close the valve ceasing flow of nitrogen through feed line 30c. Valve 66c is closed. Valve 106 remains open throughout this operation to prevent excessive pressure build up in the reaction chamber 22.

Step 42: The cleavage reaction step is permitted to occur (2-5) minutes.

Cup drive 29 is directed to slow cup speed to

1200 rpm. Valve 56 is opened purging the reaction cup 21 and chamber 22 with nitrogen. Then valve 146 is closed. Following purge valve 56 is closed shutting off nitrogen flow to the reaction cup 21 and chamber 22 permitting the cleavage reaction to take place in an inert gas atmosphere. As described the PTC derivative formed during the coupling reaction is cleaved from the protein/peptide chain as an ATZ derivative of the amino acid during this reaction.

Step 43: Restrictive vacuum is applied to the reaction cup 21 and chamber 22.

While the reaction cup 21 is spinning at low speed, valve 38 is opened applying restricted vacuum to the reaction cup 21 and chamber 22 to initiate drying of the reaction product within the reaction cup 21. At the same time nitrogen valve 56 is opened permitting a nitrogen flow through the reaction cup 21. (Valve 39 is closed, if used, ceasing vacuum application to the fraction collector 155.)

Step 44: Full vacuum is applied to the reaction chamber 22. Vacuum valves 38 and 36 are opened in order to apply full vacuum to reaction chamber 22 via vacuum line 110, 109 to continue the drying of the reaction

product. The system control means is provided with a time based control.

Adjustable time based control over the drying process in this and the previous step is provided in the sequencer system control means to assure tht the reaction product is not over dried.

Step 45: Pause

Valves 36 and 38 are closed discontinuing application of the vacuum to the cup 21 and chamber 22. Valve 56 is opened permitting nitrogen to flow into the reaction cup for pressure equilibrium purposes outlined in Step 5. Valve 56 is closed ceasing application of nitrogen to the reaction cup 21 and chamber 22.

Step 46: Solvent reservoir S3 (butyl chloride) is vented.

Valve 74h is opened to slowly vent reservoir S4 to the atmosphere to drop the total pressure inside reservoir S3 below that of the pressurized nitrogen to be applied. Valve 165 is opened connecting the interior of the fraction collector 155 to the exhaust flow 41.

Step 47: Solvent reservoir S4 is pressurized.

Valve 66d is opened permitting nitrogen to flow through lines 59 and 64 into the reservoir of solvent S4 establishing a dynamic equilibrium pressure condition inside the reservoir S4 as previously described.

Step 48: Solvent S4 is delivered and extraction of reaction product takes place.

Valves 66d and 74h remain open so that nitrogen continues to flow into solvent reservoir S4. Valve 56 is opened permitting nitrogen to flow into the reaction cup 21 and through chamber 22. Valve 161 is opened applying vacuum to valve 90b opening valve unit 90b and connecting the effluent line 151 with collector line 156 to the fraction collector 155. Valve 89g is actuated applying vacuum through line 83g to open valve unit 103c permitting solvent to flow from the reservoir S4 through feed line 30c into the reaction cup 21. Solvent S4 (butyl chloride) flows over the reagent and sample on the interior wall of the reaction cup 21 and washes the reactants within the cup to extract cleaved ATZ derivative of the first amino acid of the protein peptide chain. The solvent flows over the bottom and up the interior wall of the reaction cup 21 due to centrifugal force, extracts the derivative, rises to the

groove 101 where it is scooped into the pick up of effluent line 151 and passed through lines 151 and 136 to the fraction collector 155.

Step 49: Delivery of the reaction product to the fraction collector and wash.

Valve 165 is closed and valve 39 or 39' is opened applying vacuum to the interior of the fraction collector 155. Nitrogen control valve 56 is open permitting pressurized nitrogen to enter the cup 21 and assist flow of solvent and extract through effluent line 151. Reaction product is transferred through effluent line 151 and collector line 156 to the delivery head in the fraction collector 155 for deposit in a collector tube. The processes and reactions involved in the performance of the conversion reaction step are completely described in the referenced patent application Serial No. 500,670 and are not considered necessary to a description of the present invention.

Valve 90b is closed and valve 90a opened through actuation of valves 161 and 162, connecting the effluent line 151 with waste line 154 to the waste bottle 153.

Step 50: Pause.

Valves 66d and 74h are closed shutting off nitrogen flow through the reservoir S4. Solvent delivery is ceased by actuating valve 89g to apply vacuum through connecting line 86d to line 83g permitting nitrogen flow to the valve unit 103d breaking the vacuum and closing valve unit 103d terminating the flow of solvent S4 into the reaction cup 21. Valve 90a remains open to insure that all excess reagents and solvents are flushed from the reaction cup 21. Valve 56 remains open flowing nitrogen through the reaction cup 21 which nitrogen assists in the removal of the excess reagents and solvents.

Step 51: Restricted vacuum is applied to the reaction cup 21.

Valve 162 is closed closing valve unit 90a and disconnecting effluent line 151 from the waste bottle 153. (Valve 39' is closed, if used, ceasing application of vacuum through the fraction collector 155.) Valve 38 is opened applying restricted vacuum to the reaction cup 21 to commence drying of the remaining sample reactants within the reaction cup 21 to evacuate the reagents and solvents held within the delivery lines 30. Valve 56 is closed ceasing flow of nitrogen through the reaction cup 21.

Step 52: Vacuum is applied to reaction chamber.

Valves 36 and 38 are opened in order to apply vacuum to the reaction chamber 22 to continue drying of the constituents within the reaction cup 21. (Valve 39' is closed, if used, ceasing application of vacuum to the interior of the fraction collector 155.) Valve 162 is closed closing valve unit 90A and disconnecting the effluent line 151 from the waste bottle 153.

At this point a first cycle in the sequential degradation of a peptide or protein is complete and steps beginning with Step 5 may be performed again, and as many times as necessary, to completely degrade the amino acid chain comprising the petide/protein. The following cup or chamber wash steps may be performed at the end of each cycle, at the end of selected cycles, or only at the end of a completed sample run.

Step 53: Reagent reservoir R4 (wash solution) is vented.

Valve 74d is opened to slowly vent reservoir R4 to the atmosphere to drop the total pressure inside the reservoir R3 below that of the pressurized nitrogen to be applied.

Step 54: Reagent reservoir R4 (wash solution) is pressurized.

Valve 68d is opened permitting nitrogen to flow through vacuum line 67d into the reagent reservoir R4 to establish a dynamic equilibrium pressure condition within the reservoir R4, as previously described with reference to Fig. 2.

Step 55: Reagent R4 (wash solution) is delivered into the reaction cup 21 to wash the contained reactants.

Cup drive 29 is controlled to spin the reaction cup 21 at low speed (1200 rpm). Valves 68d and 74d remain open so that nitrogen continues to flow into reservoir R4. Valve 56 is opened permitting pressurized nitrogen to flow through the reaction cup 21 and chamber 22. Valve 146 is opened connecting drain port 145 through waste line 147 to the waste bottle 153. No vacuum is applied to the reaction chamber 22 or the cup 21. Valve 89h is actuated applying vacuum through connecting line 83h to open valve unit 105d permitting reagent R4 to flow from the reservoir for R4 through feed line 30d into the reaction cup 21. Reagent R4 (wash solution) is delivered into the interior of the reaction

cup 21. Note that wash solution may also be removed from the interior of the reaction cup to waste bottle 153 through effluent line 151, as directed by valves 90a and 90b. The wash solution entering the cup flows over the reaction product held within the cup (as described) and is continued to be delivered until wash solution overflows through the gap between the upper portion of the reaction cup and the support plug 143. Wash solution is sprayed from the upper portion of the reaction cup against the interior walls of the reaction chamber 22. The wash solution flows down over the interior surfaces of the reaction chamber 22 to the floor of the reaction chamber to drain port 145 through which the wash solution is removed as assisted by pressurized nitrogen flow therethrough. Reagent R4 (wash solution) delivery is ceased by actuating valve 89h to connect line 83h with nitrogen line 85d permitting nitrogen to break the vacuum and close valve unit 105d shutting off the flow of reagent R4 from its reservoir to the reaction cup 21. Valve unit 162 is controlled to open valve 90a connecting effluent line 151 with waste line 154 to waste bottle 153 to remove remaining wash solution from the interior of the reaction cup 21, as previously described. When wash solution has been removed from the reaction cup 21 and chamber 22 valves 90a and 146 are closed.

Step 56: Repeat of steps 53, 54 and 55.

The steps 53, 54 and 55 can be repeated as many times as desired to assure complete washing of the reaction product, reaction cup 21 and reactions chamber 22 are accomplished.

Furthermore the wash steps 53-55 can be modified as follows to accomplish complete washing:

(1) Solvent S_2 (ethylacetate) can be used in place of reagent R_4 (wash solution) in any one or more of the repeated wash steps;

(2) The reaction cup 21 can be stopped, and filled with wash solution while stopped to soak the interior of the cup and the support plug 143 with the selected wash solution, after which the cup is restarted and controlled to spin at high speed (1800) to complete the wash step, in a manner described in step 55.

Step 57: The reaction cup, reaction product and reaction chamber are dried.

Valve 56 continues to remain open applying

pressurized nitrogen to the reaction cup 21 and chamber 22. Vacuum valves 38 and 36 are opened in order applying first restricted vacuum and then full vacuum to the vacuum chamber through port 108 to apply a flow of nitrogen through the reaction cup 21 outwardly through the gap between the reaction cup and the support plug 143 and through the reaction chamber 22 to port 108 to dry the interior of the reaction cup including contained reactants, and the interior of the reaction chamber. Following drying of the reaction cup and reaction chamber valves 38 and 36 are closed, then valve 56 is closed leaving an inert gas atmosphere within the reaction chamber 22.

As mentioned earlier, the above discussed process with the exception of the wash steps 53-57 is only one cycle which extracts one amino acid unit from the peptide/protein. N-cycles are required to ascertain the entire amino acid sequence of the N-amino acid chain comprising the protein or peptide sample. Each cycle as described takes approximately 1.0 hour with a yield in each cycle upwards of 94%, wherein the yield is defined by the ratio of the volumetric amount of amino acid unit actually realized to the volumetric amount expected.

While the invention has been described with respect to a preferred embodiment, i.e., a best mode, it will be

apparent to those skilled in the art that various modifications and improvements may be made without departing from the inventive concepts of the described invention. For instance, the present invention may be used in synthesis processes, such as peptide synthesis.

WHAT IS CLAIMED IS:

1. An apparatus for performing chemical processes comprising:

a reaction cell having an interior space for contain chemical reactions;

a reaction chamber providing an interior volume enclosing said reaction cell;

means for selectively introducing fluids into said reaction cell;

means for selectively introducing inert gas into said reaction cell;

means for selectively withdrawing product or withdrawing waste material include(ing ?) a fluid or gas from said reaction cell;

means for withdrawing waste material from said reaction chamber.

2. The apparatus of claim 1 wherein said reaction chamber provides a fluid type seal between said defined interior volume and the surrounding environment.

3. The apparatus of claim 2 additionally including means for evacuating the interior volume of said reaction chamber.

4. The apparatus of claim 3 wherein said evacuating means comprises means for providing a first level of vacuum and means for providing a second level of vacuum.

5. The apparatus of claim 1 wherein said means for introducing inert gas introduces said inert gas to the bottom of said reaction cell.

6. The apparatus of claim 1 wherein said means for selectively introducing fluids into said reaction cell additionally comprises means for introducing sample into said reaction cell.

7. The apparatus of claim 6 wherein said means for introducing said sample automatically introduces sample into a fluidic conduit comprising said fluid introducing means.

8. The apparatus of claim 7 wherein said fluid conduit delivers buffer solution to said reaction cell.

9. The apparatus of claim 6 wherein the said sample introduction means is positioned within the reaction chamber and provides a port exposed to the exterior of said reaction chamber for receiving sample.

10. The apparatus of claim 9 additionally comprising temperature control means for controlling the temperature environment of the interior volume of said reaction chamber and said reaction cell.

11. The apparatus of claim 10 wherein said temperature control means additionally provides temperature control of fluidic control elements of said means for withdrawing product from said reaction cell.

12. The apparatus of claim 10 wherein said temperature control means additionally provides temperature control of fluidic control elements of said means for selectively introducing inert gas.

13. The apparatus of claim 10 wherein said temperature control means additionally provides temperature control of fluidic control elements of said means for withdrawing waste material from said reaction chamber.

14. The apparatus of claim 10 wherein said

temperature control means additionally provides temperature control of fluidic control elements of said means for introducing inert gas.

15. The apparatus of claim 10 wherein said temperature control means additionally provides temperature control of said means for introducing sample.

16. The apparatus of claim 10 wherein said reaction cell is centrally located within said temperature control reaction chamber.

17. The apparatus of claim 3 wherein said evacuating means and said means for withdrawing waste material for said reaction chamber cooperatively communicate with said reaction chamber.

18. The apparatus of claim 5 additionally comprising means for spinning said reaction cell said spinning means and said reaction chamber providing a fluid type seal for the interior volume of said reaction chamber.

19. The apparatus of claim 1 wherein said means for selectively introducing fluids provides means for introducing wash solution to the interior of said reaction cell and said means for withdrawing waste

material from said reaction chamber provides means for removal of said wash solution.

20. The apparatus of claim 19 additionally comprising a body member extending into the interior of said reaction chamber and said reaction cell to reduce the interior volumes thereof, said body providing means for support of elements of said fluid, product, and waste material introduction and withdrawal means, said body member comprising a longitudinal channel for receiving wash solution which communicates with vertical channels for receiving fluidic means to provide for contaminant and residue removal.

21. The apparatus of claim 1 wherein said means for introducing fluids into said reaction cell communicates with fluidic reservoir means, said reservoir means comprising vent means which vent said fluidic reservoirs to the atmosphere through a vent manifold, said vent means being organized in a manner to assure vapors from reactants contained in said reservoirs cannot react and form contaminant or residual materials within said vent manifold.

22. A process for performing a chemical reaction in an apparatus comprising a reaction cell enclosed within a reaction chamber comprising the steps of:

(1) introducing reactants into the reaction cell;

(2) performing the chemical reaction;

(3) removing the product of the chemical reaction from the reaction cell;

(4) washing the reaction cell by performing the steps of:

Step 1, filling the reaction cell with wash solution until the wash solution overflows the reaction cell;

Step 2, spinning the reaction cell to spray wash solution on the interior surfaces of the reaction chamber;

Step 3, removing wash solution from the bottom of the reaction chamber.

23. The process of claim 22 additionally comprising the step of drying the reaction cell and reaction chamber by evacuating the reaction chamber through application of vacuum and by introducing inert gas into the interior of the reaction cell.

24. A process of performing a chemical reaction in an apparatus comprising a reaction cell enclosed within a reaction chamber comprising the steps of:

(1) introducing reactants into the reaction cell;

(2) performing the chemical reaction;

(3) drawing the product of the chemical reaction by performing the steps of:

Step 1, evacuating the reaction chamber by applying vacuum to the interior of the reaction chamber; and

Step 2, introducing inert gas into the interior of the reaction cell so that inert gas flows over the reactants and reaction product contained within the reaction cell and into the reaction chamber where the inert gas is removed through evacuation.

(4) removing dried or semi-dried product from the reaction cell.

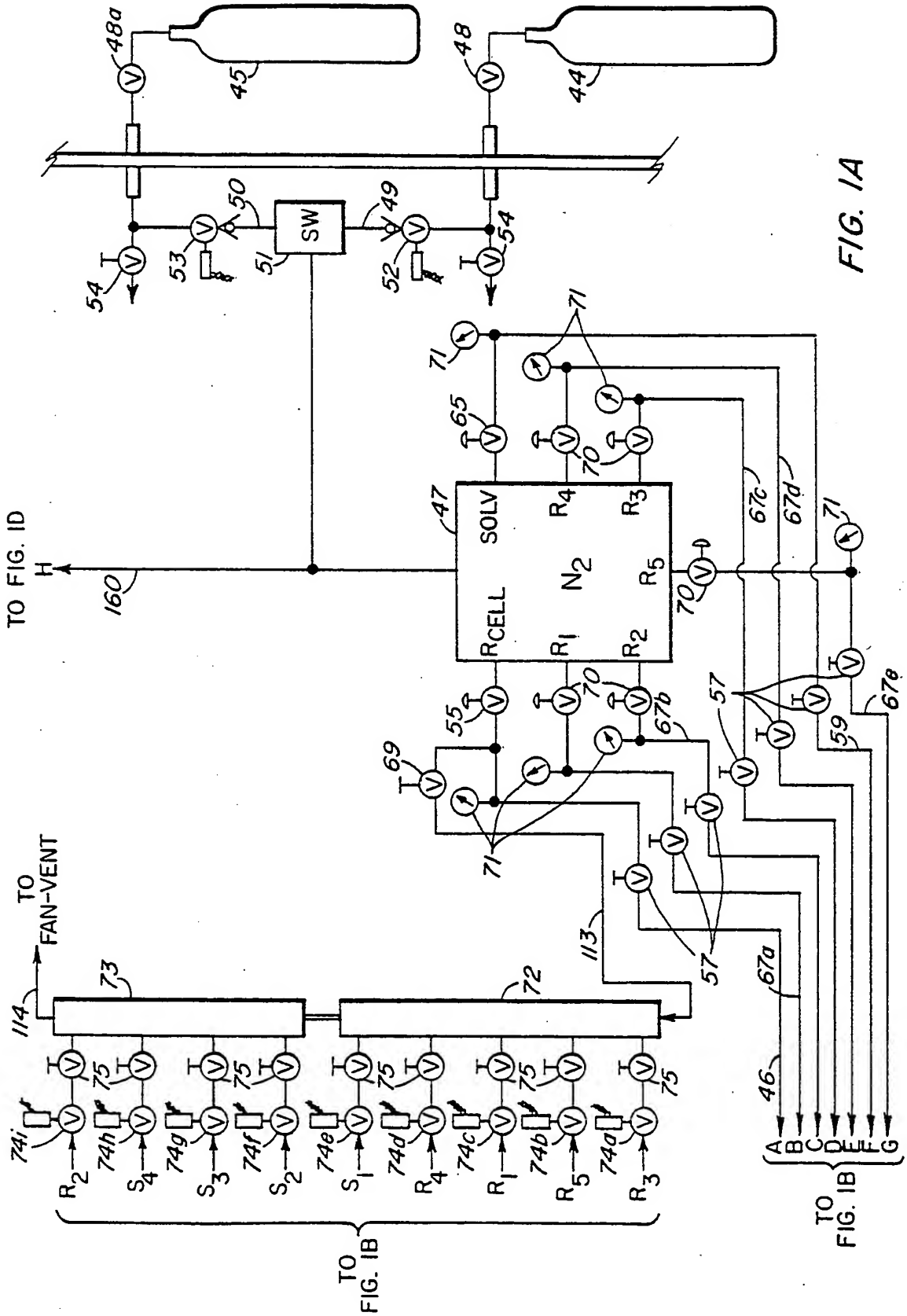
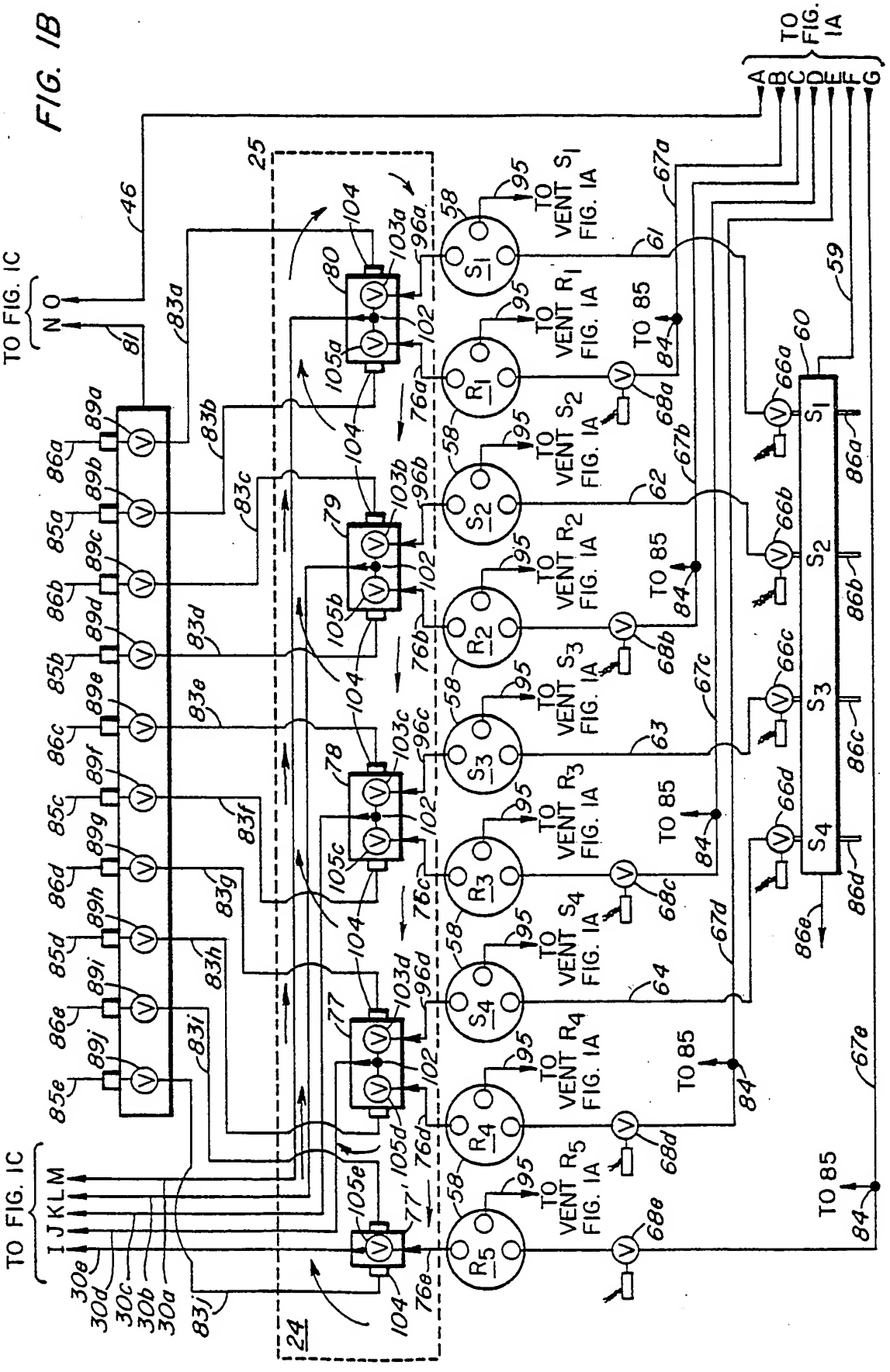

48a
45
48
44
SW
50
49
53
51
52
54
54
71
65
70
47
SOLV
R4
R3
RCELL
N2
R1
R2
R5
67c
67d
71
70
160
TO FIG. ID
H
55
69
71
67b
57
59
67a
113
TO FAN-VENT
114
73
72
75
74i
74h
74g
74f
74e
74d
74c
74b
74a
R2
S4
S3
S2
S1
R4
R1
R5
R3
TO FIG. 1B
46
A
B
C
D
E
F
G
TO FIG. 1B

FIG. 1A

FIG. 1B


TO FIG. IC
TO FIG. IA
TO VENT S1 FIG. IA
TO VENT R1 FIG. IA
TO VENT S2 FIG. IA
TO VENT R2 FIG. IA
TO VENT S3 FIG. IA
TO VENT R3 FIG. IA
TO VENT S4 FIG. IA
TO VENT R4 FIG. IA
TO VENT R5 FIG. IA
TO 85
25
24

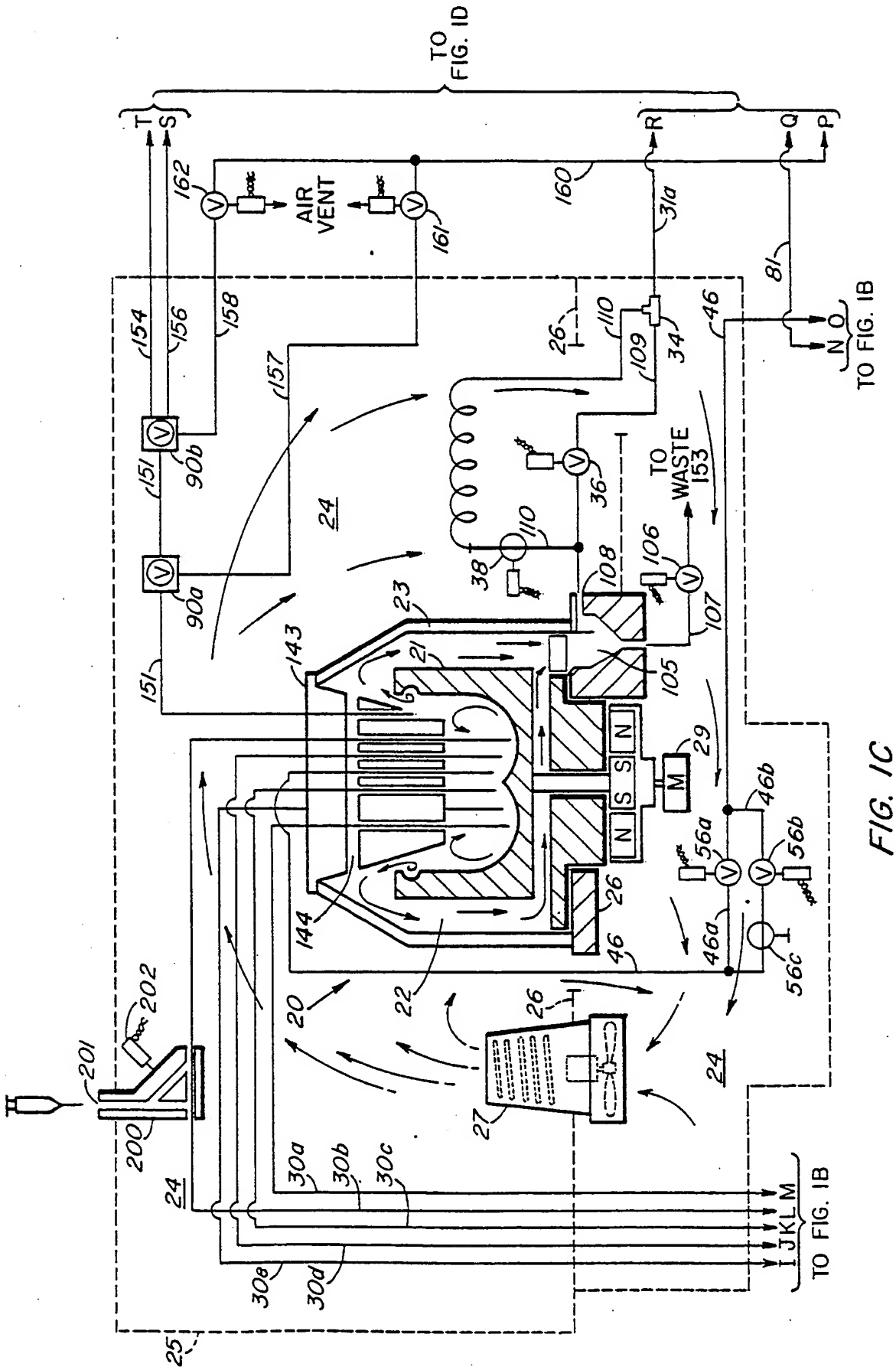

TO FIG. ID
T
S
R
Q
P
AIR VENT
TO WASTE 153
N O
TO FIG. 1B
I J K L M
TO FIG. 1B
N
S
S
N
M

FIG. 1C

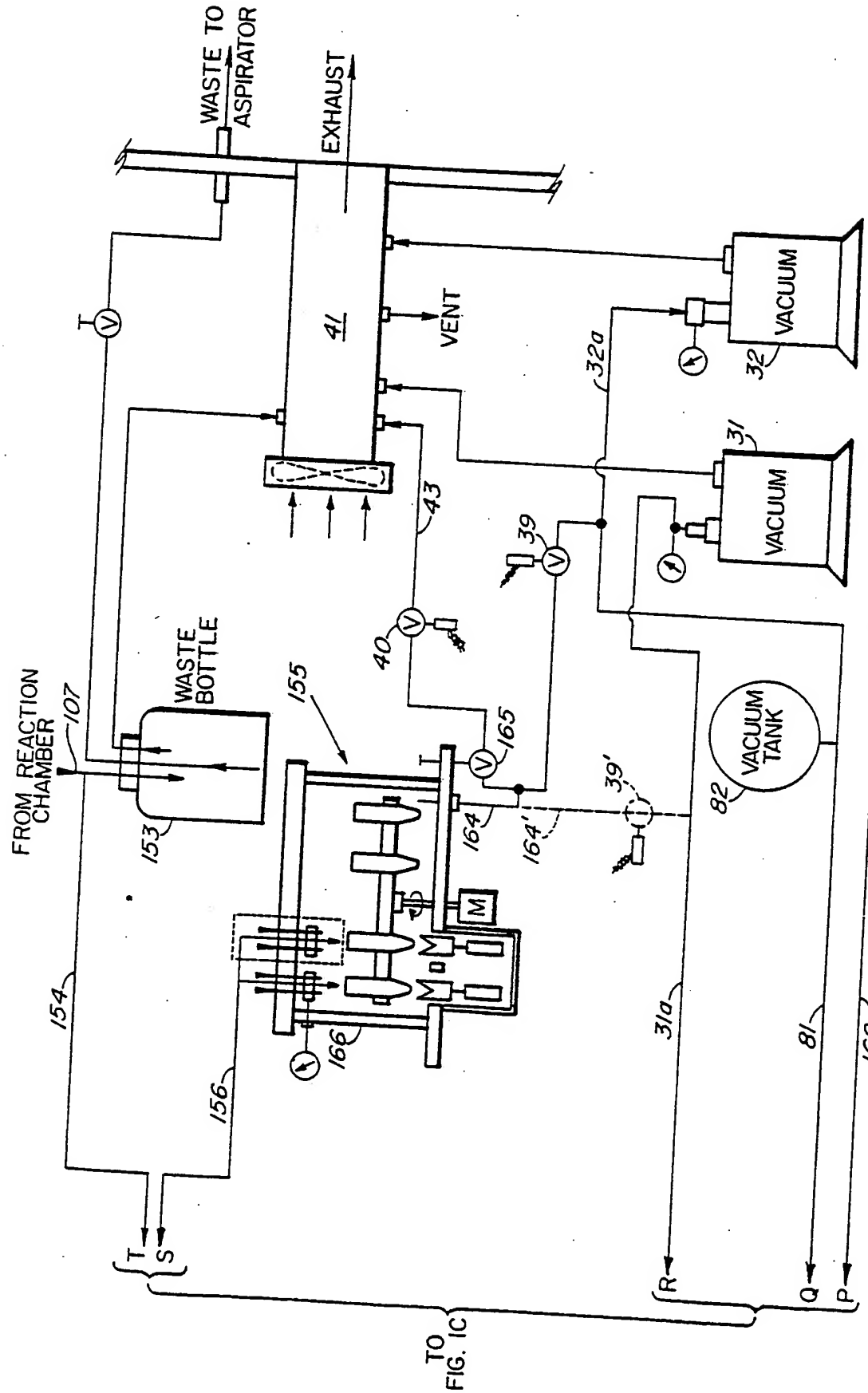

WASTE TO ASPIRATOR
EXHAUST
VENT
41
43
VACUUM
32
32a
31
39
40
165
155
WASTE BOTTLE
FROM REACTION CHAMBER
107
153
164
164'
39'
VACUUM TANK
82
M
166
154
156
31a
81
160
T
S
R
Q
P
H
TO FIG. IA
TO FIG. IC

FIG. ID

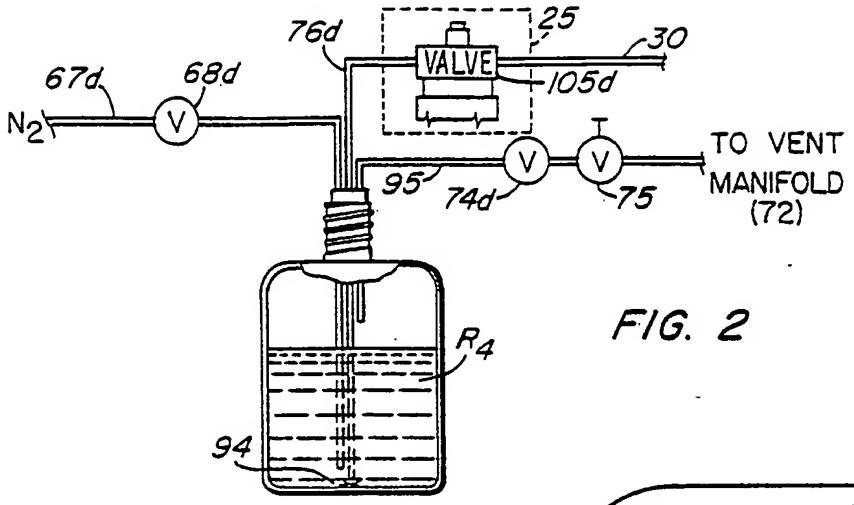

76d
25
30
VALVE
105d
67d
68d
N_2
V
V
V
TO VENT MANIFOLD (72)
95
74d
75
R_4
94

FIG. 2

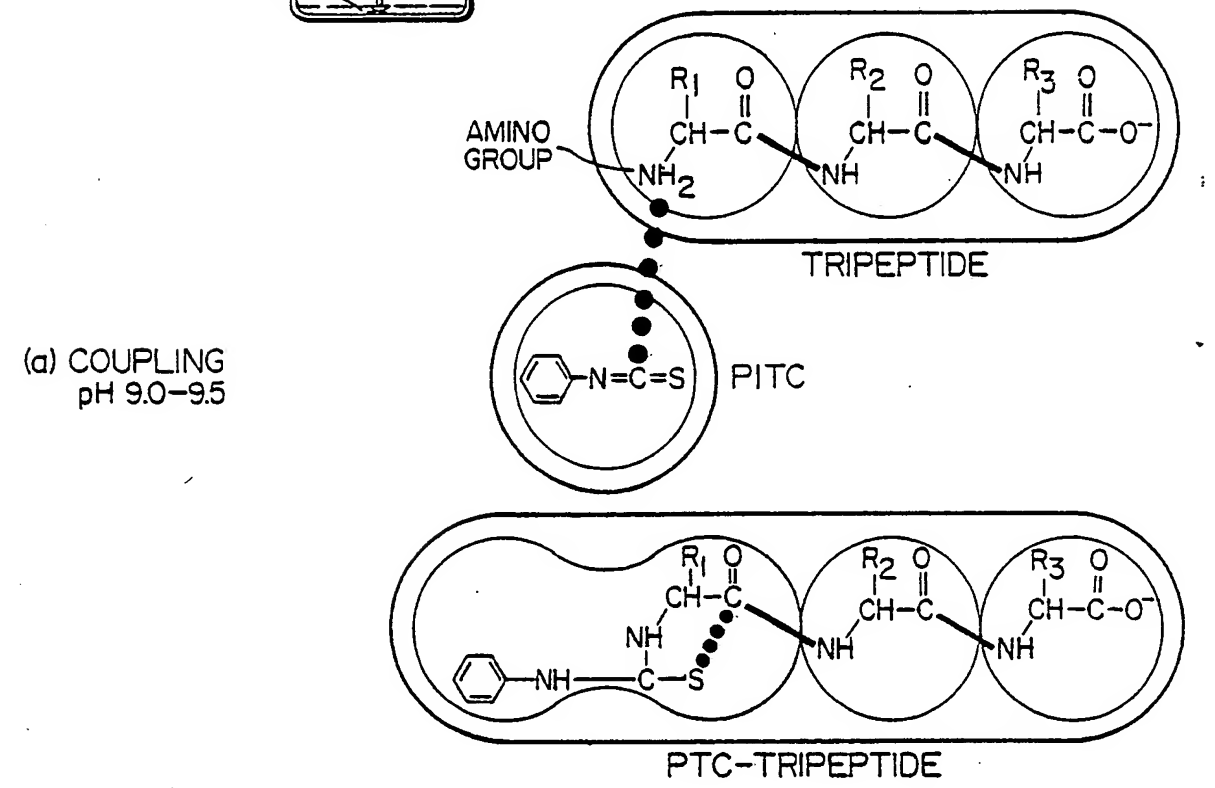

AMINO GROUP
NH_2
TRIPEPTIDE
(a) COUPLING pH 9.0–9.5
PITC
PTC-TRIPEPTIDE
(b) CLEAVAGE ANHYDROUS ACID
ATZ OF FIRST AMINO ACID
DIPEPTIDE
(c) CONVERSION AQUEOUS ACID
PTH FIRST AMINO ACID
READY FOR NEXT CYCLE

FIG. 3

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 86/02010

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[4]: G 01 N 35/00; B 01 J 19/00

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[4]	B 01 J; G 01 N

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X	WO, A, 84/02776 (BECKMAN INSTRUMENTS) 19 July 1984 see page 1, lines 5-7; page 8, line 12 - page 13, line 4; figure	1,3,5,6,8
A	--	22-24
X	US, A, 3725010 (H.A. PENHASI) 3 April 1973 see abstract; column 4, line 68 - column 16, line 23; figures 1A-3 (cited in the application)	1-3,5,6,8, 10,14,17-20
A	--	22-24
A	US, A, 4252769 (L.E. HOOD et al.) 24 February 1981 (cited in the application)	
	------	

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
17th December 1986	29 JAN 1987
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	M. VAN MOL

Form PCT/ISA/210 (second sheet) (January 1985)

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON

INTERNATIONAL APPLICATION NO. PCT/US 86/02010 (SA 14710)

This Annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 15/01/87

The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A- 8402776	I9/07/84	JP-T- 60500348	14/03/85
		EP-A- 0143789	12/06/85
US-A- 3725010	03/04/73	None	
US-A- 4252769	24/02/81	None	